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Anti A-beta antibodies and their use

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### Anti-A $\beta$ antibodies and their use

The present invention relates to antibody molecules capable of specifically recognizing two regions of the  $\beta$ -A4 peptide, wherein the first region comprises the amino acid sequence AEFRHDSGY as shown in SEQ ID NO: 1 or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLFFAEDVG as shown in SEQ ID NO: 2 or a fragment thereof. Furthermore, nucleic acid molecules encoding the inventive antibody molecules and vectors and hosts comprising said nucleic acid molecules are disclosed. In addition, the present invention provides for compositions, preferably pharmaceutical or diagnostic compositions, comprising the compounds of the invention as well as for specific uses of the antibody molecules, nucleic acid molecules, vectors or hosts of the invention.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturers specifications, instructions, etc.) are hereby incorporated by reference.

About 70% of all cases of dementia are due to Alzheimer's disease which is associated with the selective damage of brain regions and neural circuits critical for cognition. Alzheimer's disease is characterized by neurofibrillary tangles in particular in pyramidal neurons of the hippocampus and numerous amyloid plaques containing mostly a dense core of amyloid deposits and defused halos.

The extracellular neuritic plaques contain large amounts of a pre-dominantly fibrillar peptide term "amyloid  $\beta$ ", "A-beta", "A $\beta$ 4", " $\beta$ -A4" or "A $\beta$ "; see Selkoe (1994), Ann. Rev. Cell Biol. 10, 373-403, Koo (1999), PNAS Vol. 96, pp. 9989-9990, US

4,666,829 or Glenner (1984), BBRC 12, 1131. This amyloid  $\beta$  is derived from "Alzheimer precursor protein/ $\beta$ -amyloid precursor protein" (APP). APPs are integral membrane glycoproteins (see Sisodia (1992), PNAS Vol. 89, pp. 6075) and are endoproteolytically cleaved within the  $A\beta$  sequence by a plasma membrane protease,  $\alpha$ -secretase (see Sisodia (1992), loc. cit.). Furthermore, further secretase activity, in particular  $\beta$ -secretase and  $\gamma$ -secretase activity leads to the extracellular release of amyloid- $\beta$  ( $A\beta$ ) comprising either 39 amino acids ( $A\beta_{39}$ ), 40 amino acids ( $A\beta_{40}$ ), 42 amino acids ( $A\beta_{42}$ ) or 43 amino acids ( $A\beta_{43}$ ); see Sinha (1999), PNAS 96, 11094-1053; Price (1998), Science 282, 1078 to 1083; WO 00/72880 or Hardy (1997), TINS 20, 154.

It is of note that  $A\beta$  has several natural occurring forms, whereby the human forms are referred to as the above mentioned  $A\beta_{39}$ ,  $A\beta_{40}$ ,  $A\beta_{41}$ ,  $A\beta_{42}$  and  $A\beta_{43}$ . The most prominent form,  $A\beta_{42}$ , has the amino acid sequence (starting from the N-terminus): DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 27). In  $A\beta_{41}$ ,  $A\beta_{40}$ ,  $A\beta_{39}$ , the C-terminal A, IA and VIA are missing. In the  $A\beta_{43}$ -form an additional threonine residue is comprised at the C-terminus of the above depicted sequence (SEQ ID NO: 27).

The time required to nucleate  $A\beta_{40}$  fibrils was shown to be significantly longer than that to nucleate  $A\beta_{42}$  fibrils; see Koo, loc. cit. and Harper (1997), Ann. Rev. Biochem. 66, 385-407. As reviewed in Wagner (1999), J. Clin. Invest. 104, 1239-1332, the  $A\beta_{42}$  is more frequently found associated with neuritic plaques and is considered to be more fibrillogenic in vitro. It was also suggested that  $A\beta_{42}$  serves as a "seed" in the nucleation-dependent polymerization of ordered non-crystalline  $A\beta$  peptides; Jarrett (1993), Cell 93, 1055-1058.

It has to be stressed that modified APP processing and/or the generation of extracellular plaques containing proteinaceous depositions are not only known from Alzheimer's pathology but also from subjects suffering from other neurological and/or neurodegenerative disorders. These disorders comprise, inter alia, Down's syndrome, Hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's



disease, ALS (amyotrophic lateral sclerosis), Creutzfeld Jacob disease, HIV-related dementia and motor neuropathy.

In order to prevent, treat and/or ameliorate disorders and/or diseases related to the pathological deposition of amyloid plaques, means and methods have to be developed which either interfere with  $\beta$ -amyloid plaque formation, which are capable of preventing A $\beta$  aggregation and/or are useful in de-polymerization of already formed amyloid deposits or amyloid- $\beta$  aggregates.

Accordingly, and considering the severe defects of modified and/or pathological amyloid biology, means and methods for treating amyloid related disorders are highly desirable. In particular, efficient drugs which either interfere with pathological amyloid aggregation or which are capable of de-polymerization of aggregated A $\beta$  are desired. Furthermore, diagnostic means are desirable to detect, inter alia, amyloid plaques.

Thus, the technical problem of the present invention is to comply with the needs described herein above.

Accordingly, the present invention relates to an antibody molecule capable of specifically recognizing two regions of the  $\beta$ -A4/A $\beta$ 4 peptide, wherein the first region comprises the amino acid sequence AEFRHDSGY (SEQ ID NO: 1) or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLVFFAEDVG (SEQ ID NO: 2) or a fragment thereof.

In context of the present invention, the term "antibody molecule" relates to full immunoglobulin molecules, preferably IgMs, IgDs, IgEs, IgAs or IgGs, more preferably IgG1, IgG2, IgG2b, IgG3 or IgG4 as well as to parts of such immunoglobulin molecules. Furthermore, the term relates to modified and/or altered antibody molecules, like chimeric and humanized antibodies. The term also relates to monoclonal or polyclonal antibodies as well as to recombinantly or synthetically generated/synthesized antibodies. The term also relates to intact antibodies as well as to antibody fragments thereof, like, separated light and heavy chains, Fab, Fab/c,

Fv, Fab', F(ab')<sub>2</sub>. The term "antibody molecule" also comprises bifunctional antibodies and antibody constructs, like single chain Fvs (scFv) or antibody-fusion proteins. Further details on the term "antibody molecule" of the invention are provided herein below.

The term "specifically recognizing" means in accordance with this invention that the antibody molecule is capable of specifically interacting with and/or binding to at least two amino acids of each of the two regions of  $\beta$ -A4 as defined herein. Said term relates to the specificity of the antibody molecule, i.e. to its ability to discriminate between the specific regions of the  $\beta$ -A4 peptide as defined herein and another, not related region of the  $\beta$ -A4 peptide or another, not APP-related protein/peptide/test-peptide. Accordingly, specificity can be determined experimentally by methods known in the art and methods as disclosed and described herein. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-tests and peptide scans. Such methods also comprise the determination of  $K_D$ -values as, inter alia, illustrated in the appended examples. The peptide scan (pepspot assay) is used routinely employed to map linear epitopes in a polypeptide antigen. The primary sequence of the polypeptide is synthesized successively on activated cellulose with peptides overlapping one another. The recognition of certain peptides by the antibody to be tested for its ability to detect or recognize a specific antigen/epitope is scored by routine colour development (secondary antibody with horseradish peroxidase and 4-chloronaphthol and hydrogenperoxide), by a chemoluminescence reaction or similar means known in the art. In the case of, inter alia, chemoluminescence reactions, the reaction can be quantified. If the antibody reacts with a certain set of overlapping peptides one can deduce the minimum sequence of amino acids that are necessary for reaction; see illustrative Example 6 and appended Table 2.

The same assay can reveal two distant clusters of reactive peptides, which indicate the recognition of a discontinuous, i. e. conformational epitope in the antigenic polypeptide (Geysen (1986), Mol. Immunol. 23, 709-715).

In addition to the pepsot assay, standard ELISA assay can be carried out. As demonstrated in the appended examples small hexapeptides may be coupled to a

protein and coated to an immunoplate and reacted with antibodies to be tested. The scoring may be carried out by standard colour development (e.g. secondary antibody with horseradish peroxidase and tetramethyl benzidine with hydrogenperoxide). The reaction in certain wells is scored by the optical density, for example at 450 nm. Typical background (=negative reaction) may be 0.1 OD, typical positive reaction may be 1 OD. This means the difference positive/negative can be more than 10 fold. Further details are given in the appended examples. Additional, quantitative methods for determining the specificity and the ability of "specifically recognizing" the herein defined two regions of the  $\beta$ -A4 peptide are given herein below.

The term "two regions of the  $\beta$ -A4 peptide" relates to two regions as defined by their amino acid sequences shown in SEQ ID NOs: 1 and 2, relating to the N-terminal amino acids 2 to 10 and 12 to 25 of the  $\beta$ -A4 peptide. The term " $\beta$ -A4 peptide" in context of this invention relates to the herein above described A $\beta$ 39, A $\beta$ 41, A $\beta$ 43, preferably to the A $\beta$ 40 and A $\beta$ 42. It is of note that the term "two regions of the  $\beta$ -A4 peptide" also relates to an "epitope" and/or an "antigenic determinant" which comprises the herein defined two regions of the  $\beta$ -A4 peptide or parts thereof. Accordingly, the term also relates to a conformational epitope or a discontinuous epitope consisting of said two regions or parts thereof; see also Geysen (1986), loc. cit. In context of this invention, a conformational epitope is defined by two or more discrete amino acid sequences separated in the primary sequence which come together on the surface when the polypeptide folds to the native protein (Sela, (1969) Science 166, 1365 and Laver, (1990) Cell 61, 553-6). The antibody molecules of the present invention are envisaged to specifically bind to/interact with a conformational epitope(s) composed of and/or comprising the two regions of  $\beta$ -A4 described herein or parts thereof as disclosed herein below. The "antibody molecules" of the present invention are thought to comprise a dual specificity to (a) an amino acid stretch comprising amino acids 2 to 10 (or (a) part(s) thereof) of  $\beta$ -A4 and (b) an amino acid stretch comprising amino acids 12 to 25 (or (a) part(s) thereof) of  $\beta$ -A4. Fragments or parts of these stretches comprise at least two, more preferably at least three amino acids. Preferred fragments or parts are in the first region/stretch of SEQ ID NO: 27 the amino acids AEFRHD, EF, EFR, FR, EFRHDSG, EFRHD or HDSG and in the

second region/stretch of SEQ ID NO: 27 an amino acid sequence HHQL, LV, LVFFAE, VFFAED VFFA, or FFAEDV.

A number of antibodies specifically recognizing A $\beta$  peptides have been described in the art. These antibodies have mainly been obtained by immunizing animals with A $\beta$ 1-40 or A $\beta$ 1-42 or fragments thereof using standard technologies. According to published data monoclonal antibodies that were generated by immunization with the complete A $\beta$  peptide (1-40 or 1-42) recognize exclusively an epitope close to the N-terminus of A $\beta$ . Examples are the antibodies BAP-1 and BAP-2 (Brockhaus, unpublished) which were generated by immunization of mice with A $\beta$ 1-40 and which recognize the amino acids 4-6 in the context of larger A $\beta$  peptides; see appended Example 7, Table 2 and Example 12, Table 6. Antibodies that recognize the middle part of A $\beta$  derive from immunizations with smaller peptides. For example, the antibody 4G8 was generated by immunization with the A $\beta$  peptide 1-24 and recognizes exclusively the sequence 17-24 (Kim, (1988) Neuroscience Research Communications 2, 121-130). Many other monoclonal antibodies have been generated by immunizing mice with A $\beta$ -derived fragments, and antibodies recognizing the C-terminal end of A $\beta$ 1-40 and A $\beta$ 1-42 are widely used to distinguish and quantitate the corresponding A $\beta$  peptides in biological fluids and tissues by ELISA, Western blot and immunohistochemistry analysis (Ida et al, (1996) J. Biol. Chem. 271, 22908-22914; Johnson-Wood et al., (1997), Proc. Natl. Acad. Sci. USA (1994), 1550-1555; Suzuki et al., (1994), Science 264, 1336-1340; Brockhaus (1998), Neuro Rep. 9, 1481-1486).

It is believed that the immunization with T-cell dependent antigens (often the poor immunogens) requires a proteolytic cleavage of the antigen in the endosomes of antigen presenting cells. The in vivo selection of high affinity antibodies after immunization is driven by the contact of helper T cells to antigen presenting cells. The antigen presenting cells only present short peptides and not polypeptides of large size. Accordingly, these cells have a complicated (but well known) machinery to endocytose antigen(s), degrade the antigen(s) in endosomes, combine selected peptides with suitable MHC class II molecules, and to export the peptide-MHC complex to the cell surface. This is where the antigen specific recognition by T cells

occurs, with the aim to provide help to maturing B cells. The B cells which receive most T cell help have the best chance to develop into antibody secreting cells and to proliferate. This shows that antigen processing by proteolysis is an important step for the generation of an high affinity antibody response in vivo and may explain the dominance of the N-terminal A $\beta$  epitope in prior art monoclonal and polyclonal antibodies derived by immunization.

In contrast, the selection of antibodies/antibody molecules of the present invention is driven by the physical adherence of Fab expressing phages to the antigen. There is no degradation of the antigen involved in this in vitro selection process. The phages which express the Fab with the highest affinity towards the antigen are selected and propagated. A synthetic library as employed in the appended examples to select for specific antibody molecules according to this invention is particularly suited to avoid any bias for continuous epitopes that is often found in libraries derived from immunized B cells.

It is of note that the prior art has not described antibody molecules recognizing two regions of A $\beta$ 4 or which specifically recognizes (a) discontinuous/conformational epitope(s).

Vaccination of transgenic mice overexpressing mutant human APP <sub>V717F</sub> (PDAPP mice) with A $\beta$ 1-42 resulted in an almost complete prevention of amyloid deposition in the brain when treatment was initiated in young animals, i. e. before the onset of neuropathologies, whereas in older animals a reduction of already formed plaques was observed suggesting antibody-mediated clearance of plaques (Schenk et al., (1999), Nature 400,173-177). The antibodies generated by this immunization procedure were reactive against the N-terminus of A $\beta$ 4 covering an epitope around amino acids 3-7 (Schenk et al., (1999), loc. cit.; WO 00/72880). Active immunization with A $\beta$ 1-42 also reduced behavioural impairment and memory loss in different transgenic models for Alzheimer's Disease (Janus et al., (2000) Nature 408, 979-982; Morgan et al., (2000) Nature 408, 982-985). Subsequent studies with peripherally administered antibodies, i. e. passive immunization, have confirmed that antibodies can enter the central nervous system, decorate plaques and induce clearance of preexisting amyloid plaques in APP transgenic mice (PDAPP mice) (Bard et al., (2000) Nat. Med. 6, 916-919; WO 00/72880). In these studies, the

monoclonal antibodies with the most potent *in vivo* and *ex vivo* efficacy (triggering of phagocytosis in exogenous microglial cells) were those which recognized A $\beta$ 4 N-terminal epitopes 1-5 (mab 3D6, IgG2b) or 3-6 (mab 10D5, IgG1). Likewise, polyclonal antibodies isolated from mice, rabbits or monkeys after immunization with A $\beta$ 1-42 displayed a similar N-terminal epitope specificity and were also efficacious in triggering phagocytosis and *in vivo* plaque clearing. In contrast, C-terminal specific antibodies binding to A $\beta$ 1-40 or A $\beta$ 1-42 with high affinity did not induce phagocytosis in the *ex vivo* assay and were not efficacious *in vivo* (WO 00/72880). Monoclonal antibody m266 (WO 00/72880) was raised against A $\beta$ 13-28 (central domain of A $\beta$ ) and epitope mapping confirmed the antibody specificity to cover amino acids 16-24 in the A $\beta$  sequence. This antibody does not bind well to aggregated A $\beta$  and amyloid deposits and merely reacts with soluble (monomeric) A $\beta$ , i. e. properties which are similar to another well-known and commercially available monoclonal antibody (4G8; Kim, (1988) Neuroscience Research Communications 2, 121-130; commercially available from Signet Laboratories Inc. Dedham, MA USA) which recognizes the same epitope.

*In vivo*, the m266 antibody was recently found to markedly reduce A $\beta$  deposition in PDAPP mice after peripheral administration (DeMattos, (2001) Proc. Natl. Acad. Sci. USA 98, 8850-8855). However, and in contrast to N-terminal specific antibodies, m266 did not decorate amyloid plaques *in vivo*, and it was therefore hypothesized that the brain A $\beta$  burden was reduced by an antibody-induced shift in equilibrium between CNS and plasma A $\beta$  resulting in the accumulation of brain-derived A $\beta$  in the periphery, firmly complexed to m266 (DeMattos, (2001) loc. cit.).

The antibodies of the present invention, by simultaneously binding to the N-terminal and central epitopes, combine the properties of an N-terminal-specific antibody and a central epitope-specific antibody in a single molecule. Since individual N-terminal-specific and central epitope-specific antibodies have been shown to reduce amyloid plaque burden in transgenic mice, antibodies with the dual epitope specificity, as described in the present invention, are considered to be more efficacious *in vivo*. It is well known that in the process of A $\beta$ 4 aggregation and amyloid deposition

conformational changes occur, and while the central epitope is easily accessible in soluble A $\beta$ 4 it appears to be hidden and less reactive in aggregated or fibrillar A $\beta$ 4. The fact that the central epitope-specific antibody m266 is efficacious *in vivo* indicates that neutralization of soluble A $\beta$ 4 may also be a critical parameter. The antibodies of the present invention, due to the dual epitope specificity, can bind to both fibrillar and soluble A $\beta$ 4 with similar efficacy, thus allowing interaction with amyloid plaques as well as neutralization of soluble A $\beta$ 4.

D Clearance of amyloid plaques *in vivo* in PDAPP mice after direct application of the antibodies to the brain is not dependent on the IgG subtype and may also involve a mechanism which is not Fc-mediated, i. e. no involvement of activated microglia in plaque clearance (Bacscai, (2001), Abstract Society for Neuroscience 31<sup>st</sup> Annual Meeting, November 10-15, 2001, San Diego). This observation is in contrast to what has been postulated in an earlier study by Bard (2000), loc. cit.

In another study antibodies raised against A $\beta$ 1-28 and A $\beta$ 1-16 peptides were found to be effective in disaggregating A $\beta$  fibrils *in vitro*, whereas an antibody specific for A $\beta$ 13-28 was much less active in this assay (Solomon, (1997) Proc. Natl. Acad. Sci. USA 94, 4109-4112). Prevention of A $\beta$  aggregation by an anti-A $\beta$ 1-28 antibody has also been reported (Solomon, (1996) Proc. Natl. Acad. Sci. USA 93, 452-455).

O The efficacy of the various antibodies in these *in vitro* assays correlates with the accessibility of their epitopes in A $\beta$ 4 aggregates. The N-terminus is exposed and N-terminal specific antibodies clearly induce de-polymerization, whereas the central region and the C-terminus are hidden and not easily accessible and thus antibodies against these epitope are much less effective.

Investigations with respect to epitope accessibility for antibodies have shown that in aggregated A $\beta$  the N-terminal epitope is exposed and reacts with the BAP-1 antibody, whereas the middle or central epitope indeed remains cryptic, i. e. no binding of the 4G8 antibody was observed. However, in monomeric A $\beta$  both epitopes are overt and are equally recognized by both prior art antibodies.

In contrast, in the present invention, it was surprisingly found that the herein described antibody molecules recognize two discontinuous amino acid sequences, i.e. a conformational epitope on the A $\beta$  peptide.

The binding area of an antibody Fab (=paratope) occupies a molecular surface of approximately 30 x 30 Å in size (Laver, Cell 61 (1990), 553-556). This is enough to contact 15 to 22 amino acid residues which may be present on several surface loops. The discontinuous epitope recognized by the inventive antibody molecules resembles a conformation in which the N-terminal (residues 2 to 10 or parts thereof) and middle A $\beta$  peptide sequences (residues 12 to 25 or parts thereof) are in close proximity. Only within this conformation, the maximum number of antigen-antibody contacts and the lowest free energy state are obtained.

Based on energetic calculations it has been suggested that a smaller subset of 5-6 residues, which are not arranged in a linear sequence but are scattered over the epitope surface, contributes most of the binding energy while surrounding residues may merely constitute a complementary array (Laver (1990) loc. cit.).

The inventive antibodies are capable of binding to aggregated A $\beta$  and strongly react with amyloid plaques in the brain of AD patients (as documented in the appended examples). In addition, they are capable of de-polymerizing/disintegrating amyloid aggregates.

The conformational epitope (composed by the two regions of A $\beta$ 4 or (a) part(s) thereof as described herein) is believed to be partially exposed in aggregated A $\beta$ . However, it is known that major part of the middle/second epitope/region alone is not freely accessible in these A $\beta$  aggregates (based on the poor reactivities of middle epitope-specific antibodies 4G8 and m266). On the other hand, and in view of the considerations mentioned above, it is likely that one or several residues of the middle region are components of the conformational epitope and, in conjunction with the residues from the N-terminal region, are accessible to the antibodies of the present invention, thereby significantly contributing to the binding energy of the antibody-A $\beta$ 4 interaction. The reactivity of the inventive antibody molecules with the



conformational epitope in aggregated A $\beta$  is therefore unique and clearly distinct from  $\alpha$ -A $\beta$ 4 antibodies described in the prior art.

The present invention, accordingly, provides for unique tools which may be employed to de-polymerize aggregated A $\beta$ -fibrils in vivo and in vitro and/or which are capable of stabilizing and/or neutralizing a conformational epitope of monomeric A $\beta$  and thereby capable of preventing the pathological A $\beta$  aggregation.

It is furthermore envisaged that the inventive antibodies bind to A $\beta$  deposits at the rim of amyloid plaques in, inter alia, Alzheimer's brain and efficiently dissolve the pathological protofibrils and fibrils.

In a preferred embodiment, the antibody molecule of the invention recognizes at least two consecutive amino acids within the two regions of A $\beta$ 4 defined herein, more preferably said antibody molecule recognizes in the first region an amino acid sequence comprising the amino acids: AEFRHD, EF, EFR, FR, EFRHDSG, EFRHD or HDSG and in the second region an amino acid sequence comprising the amino acids: HHQL, LV, LVFFAE, VFFAED or VFFA, FFAEDV.

It is particularly preferred that the antibody molecule of the invention comprises a variable V<sub>H</sub>-region as encoded by a nucleic acid molecule as shown in SEQ ID NO: 3, 5 or 7 or a variable V<sub>H</sub>-region as shown in the amino acid sequences depicted in SEQ ID NOs: 4, 6 or 8.

The sequences as shown in SEQ ID NOs: 3 and 4 depict the coding region and the amino acid sequence of the V<sub>H</sub>-region of the inventive, parental antibody MSR-3, the sequences in SEQ ID NOs: 5 and 6 depict the coding region and the amino acid sequence of the V<sub>H</sub>-region of the inventive, parental antibody MSR-7 and SEQ ID NOs: 7 and 8 depict the coding region and the amino acid sequence of the V<sub>H</sub>-region of the inventive, parental antibody MSR-8. Accordingly, the invention also provides for antibody molecules which comprise a variable V<sub>L</sub>-region as encoded by a nucleic acid molecule as shown in a SEQ ID NO selected from the group consisting of SEQ ID NO: 9, 11 or 13 or a variable V<sub>L</sub>-region as shown in the amino acid sequences depicted in SEQ ID NOs: 10, 12 or 14. SEQ ID NOs: 9 and 10 correspond to the V<sub>L</sub>-

region of MSR-3, SEQ ID NOs: 11 and 12 correspond to the V<sub>L</sub>-region of MSR-7 and SEQ ID NOs: 13 and 14 correspond to the V<sub>L</sub>-region of MSR-8.

As illustrated in the appended examples, the parental antibodies MSR-3, -7 and -8, are employed to further generate optimized antibody molecules with even better properties and/or binding affinities. The corresponding strategy is exemplified and shown in the appended examples.

The optimization strategy as illustrated in the appended examples leads to a plurality of inventive, optimized antibodies. These optimized antibodies share with their parental antibodies the CDR-3 domain of the V<sub>H</sub>-region. Whereas the original framework region (as shown in appended Figure 1) remains the same, in the maturized/optimized antibody molecules, CDR1, CDR2 and/or V<sub>L</sub> CDR3-regions are changed. Illustrative, modified sequence motives for optimized antibody molecules are shown in appended table 1. Accordingly, within the scope of the present invention are also optimized antibody molecules which are derived from the herein disclosed MSR-3, -7 and -8 and which are capable of specifically reacting with/specifically recognizing the two regions of the  $\beta$ -A4 peptide as defined herein. In particular, CDR-regions, preferably CDR1s, more preferably CDR1s and CDR2s, most preferably CDR1s, CDR2s and CDR3s as defined herein may be employed to generate further inventive antibodies/antibody molecules, inter alia, by CDR-grafting methods known in the art; see Jones (1986), Nature 321, 522-515 or Riechmann (1988), Nature 332, 323-327. Most preferably the inventive antibodies are derived from the parental antibodies as disclosed herein and share, as disclosed above, the CDR-3 domain of the V<sub>H</sub>-region with at least one of said parental antibodies.

It is preferred that the antibodies/antibody molecules of the invention are characterized by their specific reactivity with  $\beta$ -A4 and/or peptides derived from said  $\beta$ -A4. For example, optical densities in ELISA-tests, as illustrated in the appended examples, may be established and the ratio of optical densities may be employed to define the specific reactivity of the parental or the optimized antibodies. Accordingly, a preferred antibody of the invention is an antibody which reacts in an ELISA-test with  $\beta$ -A4 to arrive at an optical density measured at 450 nm that is 10 times higher than the optical density measured without  $\beta$ -A4, i. e. 10 times over background. If the optical reading is performed after a certain time, e. g. 5 minutes, a signal over background ratio of 10 is obtained with the antibodies of the present invention.

In a particular preferred embodiment, the inventive antibody molecule comprises at least one CDR3 of an  $V_L$ -region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 15, 17 or 19 or at least one CDR3 amino acid sequence of an  $V_L$ -region as shown in SEQ ID NOs: 16, 18 or 20 and/or said antibody molecule comprises at least one CDR3 of an  $V_H$ -region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 21, 23 or 25 or at least one CDR3 amino acid sequence of an  $V_H$ -region as shown in SEQ ID NOs: 22, 24 or 26. Most preferred are antibodies comprising at least one CDR3 of an  $V_H$ -region as defined herein. The CDR-3 domains mentioned herein above relate to the inventive, illustrative parental antibody molecules MSR-3, -7, or -8. However, as illustrated in the appended table 1, maturized and/or optimized antibody molecules obtainable by the methods disclosed in the appended examples may comprise modified CDR1, CDR2 and CDR3 regions; see table 1. Accordingly, the antibody molecule of the invention is preferably selected from the group consisting of MSR-3, -7 and -8 or an affinity-maturized version of MSR-3, -7 or -8. Affinity-maturized versions of MSR-3, -7 and -8 comprise, inter alia, antibody molecules comprising CDR1, CDR2 and/or CDR3 regions as shown in table 1. Most preferably, the antibody of the invention comprises at least one CDR, preferably a CDR1, more preferably a CDR2, most preferably a CDR3 as shown in the appended table 1. It is of note that affinity-maturation techniques are known in the art, described in the appended examples and, inter alia, in Knappik (2000), J. Mol. Biol. 296, 55; Krebs (2000), J. Imm. Meth. 254, 67-84; WO 01/87337; WO 01/87338; US 6,300,064; EP 96 92 92 78.8 and further references cited herein below.

In a more preferred embodiment of the invention, the antibody molecule is a full antibody (immunoglobulin, like an IgG1, an IgG2, an IgG2b, an IgG3, an IgG4, an IgA, an IgM, an IgD or an IgE), an F(ab)-, Fabc-, Fv-, Fab'-, F(ab')<sub>2</sub>- fragment, a single-chain antibody, a chimeric antibody, a CDR-grafted antibody, a bivalent antibody-construct, an antibody-fusion protein or a synthetic antibody.

As illustrated in the appended examples, the inventive antibodies/antibody molecules can readily be recombinantly constructed and expressed. Preferably, the

antibody molecule of the invention comprises at least one, more preferably at least two, preferably at least three, more preferably at least four, more preferably at least five and most preferably at least six CDRs of the herein defined MSR-3, MSR-7 or MSR-8 parental antibodies or of affinity-maturized/optimized antibodies derived from said parental antibodies. The person skilled in the art can readily employ the information given in the appended examples to deduce corresponding CDRs of the parental as well as the affinity optimized antibodies. Examples of optimized antibodies which have been obtained by maturation/optimization of the parental antibodies are, inter alia, shown in appended table 1.

Inventive antibody molecules can easily be produced in sufficient quantities, inter alia, by recombinant methods known in the art, see, e.g. Bentley, Hybridoma 17 (1998), 559-567; Racher, Appl. Microbiol. Biotechnol. 40 (1994), 851-856; Samuelsson, Eur. J. Immunol. 26 (1996), 3029-3034.

In a most preferred embodiment of the invention, the inventive antibody molecule is an antibody molecule wherein the least two regions of the  $\beta$ -A4 to be specifically recognized by said antibody form a conformational epitope or a discontinuous epitope; see Geysen (1986), loc. cit.; Ghoshal (2001), J. Neurochem. 77, 1372-1385; Hochleitner (2000), J. Imm. 164, 4156-4161; Laver (1990), loc. cit.. The term "discontinuous epitope" means in context of the invention non-linear epitopes that are assembled from residues from distant portions of the polypeptide chain. These residues come together on the surface when the polypeptide chain folds into a three-dimensional structure to constitute a conformational epitope. The present invention provides for preferred, unexpected epitopes within  $\beta$ -A4, which result in the inventive generation of specific antibody molecules, capable of specifically interacting with these epitopes. These inventive antibodies/antibody molecules provide the basis for increased efficacy, and a reduced potential for side effects

Theoretically, in soluble  $\beta$ -A4 (monomeric/oligomeric) both the N-terminal and the middle epitopes are accessible for antibody interaction and antibody molecules of the present invention may either bind to the N-terminal or middle epitope separately, but under these conditions maximum affinity will not be obtained. However, it is more

likely that an optimal contact to the antibody paratope will be attained by simultaneous binding to both epitopes, i. e. similar to the interaction with aggregated  $\beta$ -A4. Thus, antibodies of the present invention are unique anti-A $\beta$  monoclonal antibodies in that they bind to aggregated  $\beta$ -A4 (via interaction with the N-terminal and middle epitope), and at the same time are also able to stabilize and neutralize the conformational epitope in soluble  $\beta$ -A4. These antibodies are distinct to prior art antibodies.

Most preferred are antibody molecules of the invention which have an affinity to A $\beta$  or defined fragments thereof with a  $K_D$  value lower than 2000 nM, preferably lower than 100 nM, more preferably lower than 10 nM, most preferably lower than 1 nM. The measurement of such affinity/affinities may be carried out by methods illustrated in the examples and known in the art. Such methods comprise, but are not limited to BIACORE<sup>TM</sup>-assays ([www.biacore.com](http://www.biacore.com)) and solid phase assays using labeled antibodies or labeled A $\beta$ .

Preferably, the antibody molecule of the invention is capable of decorating/reacting with amyloid plaques in in vitro brain sections from patients suffering from amyloid-related disorders, like Alzheimer's disease. It is preferred that the inventive antibody/antibody molecules may prevent A $\beta$ -aggregation in vivo as well as in in vitro assays, as illustrated in the appended examples. Similarly, the antibody molecules of the present invention are preferred to de-polymerize A $\beta$ -aggregate in vivo and/or in in vitro assays shown in the examples.

The invention also provides for a nucleic acid molecule encoding an inventive antibody molecule as defined herein.

Said nucleic acid molecule may be a naturally nucleic acid molecule as well as a recombinant nucleic acid molecule. The nucleic acid molecule of the invention may, therefore, be of natural origin, synthetic or semi-synthetic. It may comprise DNA, RNA as well as PNA and it may be a hybrid thereof.

It is evident to the person skilled in the art that regulatory sequences may be added to the nucleic acid molecule of the invention. For example, promoters, transcriptional enhancers and/or sequences which allow for induced expression of the polynucleotide of the invention may be employed. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g., by Gossen and Bujard (Proc. Natl. Acad. Sci. USA 89 (1992), 5547-5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62), or a dexamethasone-inducible gene expression system as described, e.g. by Crook (1989) EMBO J. 8, 513-519.

Furthermore, said nucleic acid molecule may contain, for example, thioester bonds and/or nucleotide analogues. Said modifications may be useful for the stabilization of the nucleic acid molecule against endo- and/or exonucleases in the cell. Said nucleic acid molecules may be transcribed by an appropriate vector containing a chimeric gene which allows for the transcription of said nucleic acid molecule in the cell. In this respect, it is also to be understood that the polynucleotide of the invention can be used for "gene targeting". In a preferred embodiment said nucleic acid molecules are labeled. Methods for the detection of nucleic acids are well known in the art, e.g., Southern and Northern blotting, PCR or primer extension.

The nucleic acid molecule(s) of the invention may be a recombinantly produced chimeric nucleic acid molecule comprising any of the aforementioned nucleic acid molecules either alone or in combination. Preferably, the nucleic acid molecule of the invention is part of a vector.

The present invention therefore also relates to a vector comprising the nucleic acid molecule of the present invention.

The vector of the present invention may be, e.g., a plasmid, cosmid, virus, bacteriophage or another vector used e.g. conventionally in genetic engineering, and may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.

Furthermore, the vector of the present invention may, in addition to the nucleic acid sequences of the invention, comprise expression control elements, allowing proper expression of the coding regions in suitable hosts. Such control elements are known

to the artisan and may include a promoter, a splice cassette, translation initiation codon, translation and insertion site for introducing an insert into the vector. Preferably, the nucleic acid molecule of the invention is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

Control elements ensuring expression in eukaryotic and prokaryotic cells are well known to those skilled in the art. As mentioned herein above, they usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions. Possible regulatory elements permitting expression in for example mammalian host cells comprise the CMV- HSV thymidine kinase promoter, SV40, RSV-promoter (Rous Sarcoma Virus), human elongation factor 1 $\alpha$ -promoter, the glucocorticoid-inducible MMTV-promoter (Moloney Mouse Tumor Virus), metallothionein- or tetracyclin-inducible promoters, or enhancers, like CMV enhancer or SV40-enhancer. For expression in neural cells, it is envisaged that neurofilament-, PGDF-, NSE-, PrP-, or thy-1-promoters can be employed. Said promoters are known in the art and, inter alia, described in Charron (1995), J. Biol. Chem. 270, 25739-25745. For the expression in prokaryotic cells, a multitude of promoters including, for example, the tac-lac-promoter or the trp promoter, has been described. Besides elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pRc/CMV, pcDNA1, pcDNA3 (In-vitro-gene), pSPORT1 (GIBCO BRL), pX (Pagano (1992) Science 255, 1144-1147), yeast two-hybrid vectors, such as pEG202 and dpJG4-5 (Gyuris (1995) Cell 75, 791-803), or prokaryotic expression vectors, such as lambda gt11 or pGEX (Amersham-Pharmacia). Beside the nucleic acid molecules of the present invention, the vector may further comprise nucleic acid sequences encoding for secretion signals. Such sequences are well known to the person skilled in the art. Furthermore, depending on the expression system used leader sequences capable of directing the peptides of the invention to a cellular compartment may be added to

the coding sequence of the nucleic acid molecules of the invention and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a protein thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusionprotein including an C- or N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the antibody molecules or fragments thereof of the invention may follow.

Furthermore, the vector of the present invention may also be an expression, a gene transfer or gene targeting vector. Gene therapy, which is based on introducing therapeutic genes into cells by ex-vivo or in-vivo techniques is one of the most important applications of gene transfer. Transgenic mice expressing a neutralizing antibody directed against nerve growth factor have been generated using the "neuroantibody" technique; Capsoni, Proc. Natl. Acad. Sci. USA 97 (2000), 6826-6831 and Biocca, Embo J. 9 (1990), 101-108. Suitable vectors, methods or gene-delivering systems for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813, Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Onodua, Blood 91 (1998), 30-36; Verzeletti, Hum. Gene Ther. 9 (1998), 2243-2251; Verma, Nature 389 (1997), 239-242; Anderson, Nature 392 (Supp. 1998), 25-30; Wang, Gene Therapy 4 (1997), 393-400; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957; US 5,580,859; US 5,589,466; US 4,394,448 or Schaper, Current Opinion in Biotechnology 7 (1996), 635-640, and references cited therein. In particular, said vectors and/or gene delivery systems are also described in gene therapy approaches in neurological tissue/cells (see, inter alia Blömer, J. Virology 71 (1997) 6641-6649) or in the hypothalamus (see, inter alia, Geddes, Front Neuroendocrinol. 20 (1999), 296-316



or Geddes, Nat. Med. 3 (1997), 1402-1404). Further suitable gene therapy constructs for use in neurological cells/tissues are known in the art, for example in Meier (1999), J. Neuropathol. Exp. Neurol. 58, 1099-1110. The nucleic acid molecules and vectors of the invention may be designed for direct introduction or for introduction via liposomes, viral vectors (e.g. adenoviral, retroviral), electroporation, ballistic (e.g. gene gun) or other delivery systems into the cell. Additionally, a baculoviral system can be used as eukaryotic expression system for the nucleic acid molecules of the invention. The introduction and gene therapeutic approach should, preferably, lead to the expression of a functional antibody molecule of the invention, whereby said expressed antibody molecule is particularly useful in the treatment, amelioration and/or prevention of neurological disorders related to abnormal amyloid synthesis, assembly and/or aggregation, like, Alzheimer's disease and the like.

Accordingly, the nucleic acid molecule of the present invention and/or the above described vectors/hosts of the present invention may be particularly useful as pharmaceutical compositions. Said pharmaceutical compositions may be employed in gene therapy approaches. In this context, it is envisaged that the nucleic acid molecules and/or vectors of the present invention may be employed to modulate, alter and/or modify the (cellular) expression and/or concentration of the antibody molecules of the invention or of (a) fragment(s) thereof.

For gene therapy applications, nucleic acids encoding the peptide(s) of the invention or fragments thereof may be cloned into a gene delivering system, such as a virus and the virus used for infection and conferring disease ameliorating or curing effects in the infected cells or organism.

The present invention also relates to a host cell transfected or transformed with the vector of the invention or a non-human host carrying the vector of the present invention, i.e. to a host cell or host which is genetically modified with a nucleic acid molecule according to the invention or with a vector comprising such a nucleic acid molecule. The term "genetically modified" means that the host cell or host comprises in addition to its natural genome a nucleic acid molecule or vector according to the invention which was introduced into the cell or host or into one of its

predecessors/parents. The nucleic acid molecule or vector may be present in the genetically modified host cell or host either as an independent molecule outside the genome, preferably as a molecule which is capable of replication, or it may be stably integrated into the genome of the host cell or host.

The host cell of the present invention may be any prokaryotic or eukaryotic cell. Suitable prokaryotic cells are those generally used for cloning like *E. coli* or *Bacillus subtilis*. Furthermore, eukaryotic cells comprise, for example, fungal or animal cells. Examples for suitable fungal cells are yeast cells, preferably those of the genus *Saccharomyces* and most preferably those of the species *Saccharomyces cerevisiae*. Suitable animal cells are, for instance, insect cells, vertebrate cells, preferably mammalian cells, such as e.g. HEK293, NSO, CHO, MDCK, U2-OSHela, NIH3T3, MOLT-4, Jurkat, PC-12, PC-3, IMR, NT2N, Sk-n-sh, CaSki, C33A. These host cells, e.g. CHO-cells, may provide posts-translational modifications to the antibody molecules of the invention, including leader peptide removal, folding and assembly of H and C chains, glycosylation of the molecule at correct sides and secretion of the functional molecule. Further suitable cell lines known in the art are obtainable from cell line depositories, like the American Type Culture Collection (ATCC). In accordance with the present invention, it is furthermore envisaged that primary cells/cell cultures may function as host cells. Said cells are in particular derived from insects (like insects of the species *Drosophila* or *Blatta*) or mammals (like human, swine, mouse or rat). Said host cells may also comprise cells from and/or derived from cell lines like neuroblastoma cell lines. The above mentioned primary cells are well known in the art and comprise, inter alia, primary astrocytes, (mixed) spinal cultures or hippocampal cultures.

In a more preferred embodiment the host cell which is transformed with the vector of the invention is a brain cell or a cell (line) derived therefrom. However, also a CHO-cell comprising the nucleic acid molecule of the present invention may be particularly useful as host. Such cells may provide for correct secondary modifications on the expressed molecules, i.e. the antibody molecules of the present invention. These modifications comprise, inter alia, glycosylations and phosphorylations.

Hosts may be non-human mammals, most preferably mice, rats, sheep, calves, dogs, monkeys or apes. Said mammals may be indispensable for developing a cure, preferably a cure for neurological and/or neurodegenerative disorders mentioned herein. Furthermore, the hosts of the present invention may be partially useful in producing the antibody molecules (or fragments thereof) of the invention. It is envisaged that said antibody molecules (or fragments thereof) be isolated from said host. It is, inter alia, envisaged that the nucleic acid molecules and or vectors described herein are incorporated in sequences for transgenic expression. The introduction of the inventive nucleic acid molecules as transgenes into non-human hosts and their subsequent expression may be employed for the production of the inventive antibodies. For example, the expression of such (a) transgene(s) in the milk of the transgenic animal provide for means to obtain the inventive antibody molecules in quantitative amounts; see inter alia, US 5, 741, 957, US 5, 304, 489 or US 5, 849, 992. Useful transgenes in this respect comprise the nucleic acid molecules of the invention, for example, coding sequences for the light and heavy chains of the antibody molecules described herein, operatively linked with promotor and/or enhancer structures from a mammary gland specific gene, like casein or beta-lactoglobulin.

The invention also provides for a method for the preparation of an antibody molecule of the invention comprising culturing the host cell described herein above under conditions that allow synthesis of said antibody molecule and recovering said antibody molecule from said culture.

The invention also related to a composition comprising an antibody molecule of the invention or produced by the method described herein above, a nucleic acid molecule encoding for the antibody molecule of the invention, a vector comprising said nucleic acid molecule or a host-cell as defined herein above and optionally, further molecules, either alone or in combination, like e.g. molecules which are capable of interfering with the formation of amyloid plaques or which are capable of depolymerizing already formed amyloid-plaques. The term "composition" as employed herein comprises at least one compound of the invention. Preferably, such a composition is a pharmaceutical or a diagnostic composition.

The composition may be in solid, liquid or gaseous form and may be, inter alia, in a form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). Said composition may comprise at least two, preferably three, more preferably four, most preferably five antibody molecules of the invention or nucleic acid molecules encoding said antibody molecules. Said composition may also comprise optimized antibodies obtainable by the methods described herein below.

It is preferred that said pharmaceutical composition, optionally comprises a pharmaceutically acceptable carrier and/or diluent. The herein disclosed pharmaceutical composition may be partially useful for the treatment of neurological and/or neurodegenerative disorders. Said disorders comprise, but are not limited to Alzheimer's disease, amyotrophic lateral sclerosis (ALS), hereditary cerebral hemorrhage with amyloidosis Dutch type, Down's syndrome, HIV-dementia, Parkinson's disease and neuronal disorders related to aging. The pharmaceutical composition of the invention is, inter alia, envisaged as potent inhibitors of amyloid plaque formation or as a potent stimulator for the de-polymerization of amyloid plaques. Therefore, the present invention provides for pharmaceutical compositions comprising the compounds of the invention to be used for the treatment of diseases/disorders associated with pathological APP proteolysis and/or amyloid plaque formation.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. It is particularly preferred that said administration is carried out by injection and/or delivery, e.g., to a site in a brain artery or directly into brain tissue. The compositions of the invention may also be administered directly to the target site, e.g., by biolistic

delivery to an external or internal target site, like the brain. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg/kg body weight per dose; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it should also be in the range of 1  $\mu$ g to 10 mg units per kilogram of body weight per minute.

Progress can be monitored by periodic assessment. The compositions of the invention may be administered locally or systemically. It is of note that peripherally administered antibodies can enter the central nervous system, see, inter alia, Bard (2000), Nature Med. 6, 916-919. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents depending on the intended use of the pharmaceutical composition. Said agents may be drugs acting on the central nervous system, like, neuroprotective factors, cholinesterase inhibitors, agonists of M1 muscarinic receptor, hormones, antioxidants, inhibitors of inflammation etc. It is particularly preferred that said pharmaceutical composition comprises further agents like, e.g. neurotransmitters and/or substitution molecules for neurotransmitters, vitamin E, or alpha-lipoic acid.

The pharmaceutical compositions, as well as the methods of the invention or the uses of the invention described infra can be used for the treatment of all kinds of diseases hitherto unknown or being related to or dependent on pathological APP aggregation or pathological APP processing. They may be particularly useful for the treatment of Alzheimer's disease and other diseases where extracellular deposits of amyloid- $\beta$ , appear to play a role. They may be desirably employed in humans, although animal treatment is also encompassed by the methods, uses and compositions described herein.

In a preferred embodiment of the invention, the composition of the present invention as disclosed herein above is a diagnostic composition further comprising, optionally, suitable means for detection. The diagnostic composition comprises at least one of the aforementioned compounds of the invention.

Said diagnostic composition may comprise the compounds of the invention, in particular and preferably the antibody molecules of the present invention, in soluble form/liquid phase but it is also envisaged that said compounds are bound to/attached to and/or linked to a solid support.

Solid supports may be used in combination with the diagnostic composition as defined herein or the compounds of the present invention may be directly bound to said solid supports. Such supports are well known in the art and comprise, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The compound(s) of the invention, in particular the peptides of the present invention, may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Appropriate labels and methods for labeling have been identified above and are furthermore mentioned herein below. Suitable methods for fixing/immobilizing said compound(s) of the

invention are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like.

It is particularly preferred that the diagnostic composition of the invention is employed for the detection and/or quantification of APP and/or APP-processing products, like amyloid- $\beta$  or for the detection and/or quantification of pathological and/or (genetically) modified APP-cleavage sides.

As illustrated in the appended examples, the compounds of the present invention, in particular the inventive antibody molecules are particularly useful as diagnostic reagents in the detection of genuine human amyloid plaques in brain sections of Alzheimer's Disease patients by indirect immunofluorescence.

It is preferred that said compounds of the present invention to be employed in a diagnostic composition are detectably labeled. A variety of techniques are available for labeling biomolecules, are well known to the person skilled in the art and are considered to be within the scope of the present invention. Such techniques are, e.g., described in Tijssen, "Practice and theory of enzyme immuno assays", Burden, RH and von Knippenburg (Eds), Volume 15 (1985), "Basic methods in molecular biology"; Davis LG, Dimer MD; Battey Elsevier (1990); Mayer et al., (Eds) "Immunochemical methods in cell and molecular biology" Academic Press, London (1987), or in the series "Methods in Enzymology", Academic Press, Inc.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, colloidal metals, fluorescent compounds, chemiluminescent compounds, and bioluminescent compounds.

Commonly used labels comprise, inter alia, fluorochromes (like fluorescein, rhodamine, Texas Red, etc.), enzymes (like horse radish peroxidase,  $\beta$ -galactosidase, alkaline phosphatase), radioactive isotopes (like  $^{32}\text{P}$  or  $^{125}\text{I}$ ), biotin, digoxigenin, colloidal metals, chemi- or bioluminescent compounds (like dioxetanes, luminol or acridiniums). Labeling procedures, like covalent coupling of enzymes or

biotinyl groups, iodinations, phosphorylations, biotinylations, etc. are well known in the art.

Detection methods comprise, but are not limited to, autoradiography, fluorescence microscopy, direct and indirect enzymatic reactions, etc. Commonly used detection assays comprise radioisotopic or non-radioisotopic methods. These comprise, inter alia, Westernblotting, overlay-assays, RIA (Radioimmuno Assay) and IRMA (Immune Radioimmunometric Assay), EIA (Enzyme Immuno Assay), ELISA (Enzyme Linked Immuno Sorbent Assay), FIA (Fluorescent Immuno Assay), and CLIA (Chemiluminescent Immune Assay).

Furthermore, the present invention provides for the use of an antibody molecule of invention, or an antibody molecule produced by the method of the invention, of a nucleic acid molecule, vector of or a host of the invention for the preparation of a pharmaceutical or a diagnostic composition for the preparation and/or treatment of a disease associated with amyloidogenesis and/or amyloid-plaque formation.

Furthermore, another inventive use of the compounds of the present invention is the use for the preparation of a pharmaceutical composition for the disintegration of  $\beta$ -amyloid plaques or passive immunization against  $\beta$ -amyloid plaque formation. As illustrated in the appended examples, the inventive antibody molecules are particularly useful in preventing A $\beta$  aggregation and in de-polymerization of already formed amyloid aggregates.

The above recited diseases associated with amyloidogenesis and/or amyloid-plaque formation comprise, but are not limited to dementia, Alzheimer's disease, motor neuropathy, Parkinson's disease, ALS (amyotrophic lateral sclerosis), scrapie, HIV-related dementia as well as Down's syndrome and neuronal disorders related to aging.

Accordingly, the present invention also provides for a method for treating, preventing and/or delaying neurological and/or neurodegenerative disorders comprising the step of administering to a subject suffering from said neurological and/or neurodegenerative disorder and/or to a subject susceptible to said neurological



and/or neurodegenerative disorder an effective amount of a antibody molecule of the invention, a nucleic acid molecule of invention and/or a composition as defined herein above.

In yet another embodiment, the present invention provides for a kit comprising at least one antibody molecule, at least one nucleic acid molecule, at least one vector or at least one host cell of the invention. Advantageously, the kit of the present invention further comprises, optionally (a) buffer(s), storage solutions and/or remaining reagents or materials required for the conduct of medical, scientific or diagnostic assays and purposes. Furthermore, parts of the kit of the invention can be packaged individually in vials or bottles or in combination in containers or multicontainer units.

The kit of the present invention may be advantageously used, inter alia, for carrying out the method of the invention and could be employed in a variety of applications referred herein, e.g., as diagnostic kits, as research tools or medical tools. Additionally, the kit of the invention may contain means for detection suitable for scientific, medical and/or diagnostic purposes. The manufacture of the kits follows preferably standard procedures which are known to the person skilled in the art.

The invention also provides for a method for the optimization of an antibody molecule as defined herein above comprising the steps of

- (a) constructing a library of diversified Fab antibody fragments derived from an antibody comprising at least one CDR3 of an  $V_H$ -region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 21, 23 or 25 or at least one CDR3 amino acid sequence of an  $V_H$ -region as shown in SEQ ID NOs: 22, 24 or 26;
- (b) testing the resulting Fab optimization library by panning against  $A\beta/A\beta_4$ ;
- (c) identifying optimized clones; and
- (d) expressing of selected, optimized clones.

The person skilled in the art can readily carry out the inventive method employing the teachings of the present invention. Optimization protocols for antibodies are known in the art. These optimization protocols comprise, inter alia, CDR walking mutagenesis as disclosed and illustrated herein and described in Yang (1995), J.

Mol. Biol. 25, 392-403; Schier (1996), J. Mol. Biol. 263, 551-567; Barbas (1996), Trends. Biotech 14, 230-34 or Wu (1998), PNAS 95, 6037-6042; Schier (1996), Human Antibodies Hybridomas 7, 97; Moore (1997), J. Mol. Biol. 272, 336.

"Panning"-techniques are also known in the art, see, e.g. Kay (1993), Gene 128, 59-65. Furthermore, publications like Borrebaeck (1995), "Antibody Engineering", Oxford University, 229-266; McCafferty (1996), "Antibody Engineering", Oxford University Press; Kay (1996), A Laboratory Manual, Academic Press provide for optimization protocols which may be modified in accordance with this invention.

The method may further comprise a step (ca), whereby the optimized clones are further optimized by cassette mutagenesis, as illustrated in the appended examples.

The method for the optimization of an antibody molecule described herein is further illustrated in the appended examples as affinity maturation of parental antibodies /antibody molecules capable of specifically recognizing two regions of the beta <sup>+</sup>-A4 peptide/ Abeta4.

Preferably, said A $\beta$ /A $\beta$ 4 in step (b) of the method described herein above is aggregated A $\beta$ /A $\beta$ 4. Said panning may be carried out (as described in the appended examples) with increased stringency of binding. Stringency may be increased, inter alia, by reducing the A $\beta$ /A $\beta$ 4 concentration or by elevating the (assay) temperature. The testing of the optimized library by panning is known to the skilled artisan and described in Kay (1993), loc. cit..

Most preferably said identification in step (c) is carried out by koff-ranking. Koff-ranking is known to the skilled artisan and described in Schier (1996), loc. cit.; Schier (1996), J. Mol. Biol. 255, 28-43 or Duenas (1996), Mol. Immunol. 33, 279-286. Furthermore, koff-ranking is illustrated in the appended examples. Accordingly, in a preferred embodiment the identification in step (c) is carried out by ranking according to the lowest K<sub>D</sub>-values, or, at least as approximation, by ranking according to the smallest off-rate constant (koff). The off-rate constant may be measured as described in the appended examples.

As mentioned herein above, the identified clones may, for further evaluation, be expressed. The expression may be carried out by known methods, inter alia, illustrated in the appended examples. The expression may, inter alia, lead to expressed Fab-fragments, scFvs, bispecific immunoglobulins, bispecific antibody molecules, Fab- and/or Fv fusion proteins, or full antibodies, like IgGs, in particular IgG1.

Optimized antibodies, in particular optimized Fabs or optimized IgGs, preferably IgG1s, may be tested by methods as illustrated in the appended examples. Such methods comprise, but are not limited to, the testing of binding affinities, the determination of  $K_D$  values, pepspot analysis, ELISA-assays, RIA-assays, CLIA-assays, (immuno-) histological studies (for example staining of amyloid plaques), depolymerization assays or antibody-dependent  $\beta$ -A4 phagocytoses.

In a preferred embodiment, the invention relates to a method for the preparation of a pharmaceutical composition comprising the steps of

- (a) optimization of an antibody according to the method described herein and illustrated in the appended examples; and
- (b) formulating the optimized antibody/antibody molecule with an physiologically acceptable carrier, as described herein above.

Accordingly, the invention also provides for a pharmaceutical composition prepared by the method disclosed herein and comprising further optimized antibody molecules capable of specifically recognizing two regions of the beta-A4 peptide/A $\beta$ 4, as described herein above.

**SEQUENCES as recited herein:****SEQ ID NO: 1****AEFRHDSGY****First region of  $\beta$ -A4 peptide****SEQ ID NO: 2****VHHQKLVFFAEDVG****Second region of  $\beta$ -A4 peptide****SEQ ID NO: 3****VH-region of MS-Roche#3 (nucleic acid sequence)**

CAGGTGCAATTGGTGGAAAGCGGCGGCCTGGTGGCAACCGGGCGGCAGC  
CTGCGTCTGAGCTGCGCGGCCTCCGGATTACCTTTAGCAGCTATGCGATGAG  
CTGGGTGCGCCAAGCCCCTGGGAAGGGTCTCGAGTGGGTGAGCGCGATTAGC  
GGTAGCGGCGGCAGCACCTATTATGCGGATAGCGTGAAAGGCCGTTTTACCAT  
TTCACGTGATAATTCGAAAAACACCCTGTATCTGCAAATGAACAGCCTGCGTGC  
GGAAGATACGGCCGTGTATTATTGCGCGCGTCTTACTCATTATGCTCGTTATTA  
TCGTTATTTTGATGTTTGGGGCCAAGGCACCCTGGTGACGGTTAGCTCAGC  
(SEQ ID NO : 3)

**SEQ ID NO: 4****VH-region of MS-Roche#3 (amino acid sequence)**

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS  
GGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARLTHYARYYRYF  
DVWGQGT LTVSS (SEQ ID NO : 4)

**SEQ ID NO: 5****VH-region of MS-Roche#7 (nucleic acid sequence)**

CAGGTGCAATTGGTGGAAAGCGGCGGCCTGGTGGCAACCGGGCGGCAGC  
CTGCGTCTGAGCTGCGCGGCCTCCGGATTACCTTTAGCAGCTATGCGATGAG  
CTGGGTGCGCCAAGCCCCTGGGAAGGGTCTCGAGTGGGTGAGCGCGATTAGC  
GGTAGCGGCGGCAGCACCTATTATGCGGATAGCGTGAAAGGCCGTTTTACCATT  
TCACGTGATAATTCGAAAAACACCCTGTATCTGCAAATGAACAGCCTGCGTGCG  
GAAGATACGGCCGTGTATTATTGCGCGCGTGGTAAGGGTAATACTCATAAGCCT  
TATGGTTATGTTTCGTTATTTTGATGTTTGGGGCCAAGGCACCCTGGTGACGGTT  
AGCTCAGC (SEQ ID NO: 5)

## SEQ ID NO: 6

VH-region of MS-Roche#7 (amino acid sequence)

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS  
GGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGKGNTHKPYGY  
VRYFDVWGQGTLVTVSS (SEQ ID NO: 6)

## SEQ ID NO: 7

VH-region of MS-Roche#8 (nucleic acid sequence)

CAGGTGCAATTGGTGGAAAGCGGCGGCCTGGTGCAACCGGGCGGCAGC  
CTGCGTCTGAGCTGCGCGGCCTCCGATTACCTTTAGCAGCTATGCGATGAG  
CTGGGTGCGCCAAGCCCCTGGGAAGGGTCTCGAGTGGGTGAGCGCGATTAGC  
GGTAGCGGCGGCAGCACCTATTATGCGGATAGCGTGAAAGGCCGTTTTACCAT  
TTCACGTGATAATTCGAAAAACACCCTGTATCTGCAAATGAACAGCCTGCGTGC  
GGAAGATACGGCCGTGTATTATTGCGCGCGTCTTCTTTCTCGTGGTTATAATGG  
TTATTATCATAAGTTTGATGTTTGGGGCCAAGGCACCCTGGTGACGGTTAGCTC  
AGC (SEQ ID NO: 7)

## SEQ ID NO: 8

VH-region of MS-Roche#8 (amino acid sequence)

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS  
GGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARLLSRGYNGYYH  
KFDVWGQGTLVTVSS (SEQ ID NO: 8)

## SEQ ID NO: 9

VL-region of MS-Roche#3 (nucleic acid sequence)

GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC  
GTGCGACCCTGAGCTGCAGAGCGAGCCAGAGCGTGAGCAGCAGCTATCTGGC  
GTGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGA  
GCAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCAC  
GGATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGGTTTATTA  
TTGCCAGCAGGTTTATAATCCTCCTGTTACCTTTGGCCAGGGTACGAAAGTTGA  
AATTAAACGTACG (SEQ ID NO: 9)

## SEQ ID NO: 10

VL-region of MS-Roche #3 (amino acid sequence)

DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA  
TGVPARFSGSGSGTDFTLTISSELPEDFAVYYCQQVYNPPVTFGQGTKVEIKRT  
(SEQ ID NO: 10)

## SEQ ID NO: 11

VL-region of MS-Roche#7 (nucleic acid sequence)

GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGCGAAC  
GTGCGACCCTGAGCTGCAGAGCGAGCCAGAGCGTGAGCAGCAGCTATCTGGC

GTGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGA  
GCAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCAC  
GGATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGACTTATTA  
TTGCTTTCAGCTTTATTCTGATCCTTTTACCTTTGGCCAGGGTACGAAAGTTGAA  
ATTAAACGTACG (SEQ ID NO. 11)

SEQ ID NO: 12

VL-region of MS-Roche#7 (amino acid sequence)  
DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA  
TGVPARFSGSGSGTDFTLTISSELPEDFATYYCFQLYSDPFTFGQGTKVEIKRT  
(SEQ ID NO : 12)

SEQ ID NO: 13

VL-region of MS-Roche#8 (nucleic acid sequence)  
GATATCGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGTCTCCGGGGCGAAC  
GTGCGACCCTGAGCTGCAGAGCGAGCCAGAGCGTGAGCAGCAGCTATCTGGC  
GTGGTACCAGCAGAAACCAGGTCAAGCACCGCGTCTATTAATTTATGGCGCGA  
GCAGCCGTGCAACTGGGGTCCCGGCGCGTTTTAGCGGCTCTGGATCCGGCAC  
GGATTTTACCCTGACCATTAGCAGCCTGGAACCTGAAGACTTTGCGACTTATTA  
TTGCCAGCAGCTTTCTTCTTTTCCTCCTACCTTTGGCCAGGGTACGAAAGTTGA  
AATTAAACGTACG (SEQ ID NO: 13)

SEQ ID NO: 14

VL-region of MS-Roche#8 (amino acid sequence)  
DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRA  
TGVPARFSGSGSGTDFTLTISSELPEDFATYYCQQLSSFPTFGQGTKVEIKRT  
(SEQ ID NO : 14)

SEQ ID NO: 15

CDR3 of V<sub>L</sub>-region of MSR-3 (nucleic acid sequence)  
|CAGCAGGTTTATAATCCTCCTGTT|  
(SEQ ID NO : 15)

SEQ ID NO: 16

CDR3 of V<sub>L</sub>-region of MSR-3 (amino acid sequence)  
QQVYNPPV (SEQ ID NO: 16)

SEQ ID NO: 17

CDR3 of V<sub>L</sub>-region of MSR-7 (nucleic acid sequence)  
|TTTCAGCTTTATTCTGATCCTTT|  
(SEQ ID NO : 17)

SEQ ID NO: 18

CDR3 of V<sub>L</sub>-region of MSR-7 (amino acid sequence)

FQLYSDPF (SEQ ID NO. 18)

SEQ ID NO: 19

CDR3 of V<sub>L</sub>-region of MSR-8 (nucleic acid sequence)

CAGCAGCTTTCTTCTTTTCTCCT  
(SEQ ID NO. 19)

SEQ ID NO: 20

CDR3 of V<sub>L</sub>-region of MSR-8 (amino acid sequence)

QQLSSFPP (SEQ ID NO: 20)

SEQ ID NO: 21

CDR of V<sub>H</sub>-region of MSR-3 (nucleic acid sequence)

CTTACTCATTATGCTCGTTATTATCGTTATTTTGATGTT  
(SEQ ID NO: 21)

SEQ ID NO: 22

CDR of V<sub>H</sub>-region of MSR-3 (amino acid sequence)

LTHYARYRYFDV (SEQ ID NO: 22)

SEQ ID NO: 23

CDR of V<sub>H</sub>-region of MSR-7 (nucleic acid sequence)

GGTAAGGGTAATACTCATAAGCCTTATGGTTATGTTCTGTTATTTTGATGTT  
(SEQ ID NO: 23)

SEQ ID NO: 24

CDR of V<sub>H</sub>-region of MSR-7 (amino acid sequence)

GKGNTHKPYGYVRYFDV (SEQ ID NO: 24)

SEQ ID NO: 25

CDR of V<sub>H</sub>-region of MSR-8 (nucleic acid sequence)

CTTCTTTCTCGTGGTTATAATGGTTATTATCAT AAGTTTGATGTT  
(SEQ ID NO. 25)

SEQ ID NO: 26

CDR of V<sub>H</sub>-region of MSR-8 (amino acid sequence)

LLSRGYNGYYHKFDV (SEQ ID NO: 26)

SEQ ID NO: 27 A $\beta$ 4 (amino acids 1 to 42)

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 27)

SEQ ID NO: 28 primer

5'-GTGGTGGTTCCGATATC-3' (SEQ ID NO: 28)

SEQ ID NO: 29 primer

5'- AGCGTCACACTCGGTGCGGCTTTCGGCTGGCCAAGAACGGTTA-3' (SEQ ID NO: 29)

SEQ ID NO: 30 primer

5'-CAGGAAACAGCTATGAC-3' (SEQ ID NO: 30)

SEQ ID NO: 31 primer

5'-TACCGTTGCTCTTCACCCC-3' (SEQ ID NO: 31)

The Figures show:

#### Figure 1 Sequence summary of HuCAL<sup>®</sup>-Fab1 Library

The numbering is according to VBASE except the gap in VL $\lambda$  position 9. In VBASE the gap is set at position 10 (*Chothia et al., 1992*). In the sequence summary all CDR3 residues which were kept constant are indicated. For the CDR3 length the residues which were varied are given in brackets (x).

A: amino acid sequence

B: DNA sequence



**Figure 2 Fab display vector pMORPH<sup>®</sup>18\_Fab**

Vector map and DNA sequence including restriction sites

**Figure 3 Fab expression vector pMORPH<sup>®</sup>x9\_Fab**

Vector map and DNA sequence including restriction sites

**Figure 4 Sequences of the parental Fab fragments MS-Roche-3, MS-Roche-7 and MS-Roche 8**

A: amino acid sequence

B: DNA sequence

**Figure 5:** Indirect immunofluorescence of amyloid-plaques from a cryostat section of human temporal cortex. The plaques were labeled with MS-R # 3.2 Fab (upper panels) and MS-R # 7.4 Fab (lower panels) at 20  $\mu\text{g/ml}$  (left panels) and 5  $\mu\text{g/ml}$  (right panels) under stringent blocking conditions. Bound MS-R Fab was revealed by goat anti-human-Cy3.

**Figure 6:** Indirect immunofluorescence of amyloid-plaques from a cryostat section of human temporal cortex. The plaques were labeled with MS-R # 3.3 IgG1 (upper panels) and MS-R # 7.12 IgG1 (lower panels) at 0.05  $\mu\text{g/ml}$  (left panels) and 0.01  $\mu\text{g/ml}$  (right panels) under stringent blocking conditions. Bound MS-R IgG1 antibody was revealed by goat anti-human (H+L)-Cy3.

**Figure 7:** Polymerization Assay. Anti-A $\beta$  antibodies prevent incorporation of biotinylated A $\beta$  into preformed A $\beta$  aggregates.

**Figure 8:** De-polymerization Assay. Anti-A $\beta$  antibodies induce release of biotinylated A $\beta$  from aggregated A $\beta$ .

The examples illustrate the invention.

## Example 1: Construction and Screening of a Human Combinatorial Antibody Library (HuCAL<sup>®</sup>-Fab 1)

### *Cloning of HuCAL<sup>®</sup>-Fab 1*

HuCAL<sup>®</sup>-Fab 1 is a fully synthetic, modular human antibody library in the Fab antibody fragment format. HuCAL<sup>®</sup>-Fab 1 was assembled starting from an antibody library in the single-chain format (HuCAL<sup>®</sup>-scFv; Knappik, (2000), *J. Mol. Biol.* 296, 57-86).

*V $\lambda$  positions 1 and 2.* The original HuCAL<sup>®</sup> master genes were constructed with their authentic N-termini: VL $\lambda$ 1: QS (CAGAGC), VL $\lambda$ 2: QS (CAGAGC), and VL $\lambda$ 3: SY (AGCTAT). Sequences containing these amino acids are shown in WO 97/08320. During HuCAL<sup>®</sup> library construction, the first two amino acids were changed to DI to facilitate library cloning (*EcoRI* site). All HuCAL<sup>®</sup> libraries contain VL $\lambda$  genes with the *EcoRV* site GATATC (DI) at the 5'-end. All HuCAL<sup>®</sup> kappa genes (master genes and all genes in the library) contain DI at the 5'-end (figure 1 A and B).

*VH position 1.* The original HuCAL<sup>®</sup> master genes were constructed with their authentic N-termini: VH1A, VH1B, VH2, VH4, and VH6 with Q (=CAG) as the first amino acid and VH3 and VH5 with E (=GAA) as the first amino acid. Sequences containing these amino acids are shown in WO 97/08320. During cloning of the HuCAL<sup>®</sup>-Fab1 library, amino acid at position 1 of VH was changed to Q (CAG) in all VH genes (figure 1 A and B).

### *Design of the CDR libraries*

*V $\kappa$ 1/V $\kappa$ 3 position 85.* Because of the cassette mutagenesis procedure used to introduce the CDR3 library (Knappik, (2000), *loc. cit.*), position 85 of V $\kappa$ 1 and V $\kappa$ 3 can be either T or V. Thus, during HuCAL<sup>®</sup>-scFv1 library construction, position 85 of V $\kappa$ 1 and V $\kappa$ 3 was varied as follows: V $\kappa$ 1 original, 85T (codon ACC); V $\kappa$ 1 library, 85T or 85V (TRIM codons ACT or GTT); V $\kappa$ 3 original, 85V (codon GTG); V $\kappa$ 3 library, 85T or 85V (TRIM codons ACT or GTT); the same applies to HuCAL<sup>®</sup>-Fab1.

*CDR3 design.* All CDR3 residues, which were kept constant, are indicated in figure 1 A and B.

**CDR3 length.** The designed CDR3 length distribution is as follows. Residues, which were varied are shown in brackets (x) in figure 1. V kappa CDR3, 8 amino acid residues (position 89 to 96) (occasionally 7-10 residues), with Q89, S90, and D92 fixed; and VH CDR3, 5 to 28 amino acid residues (position 95 to 102) (occasionally 4-28), with D101 fixed.

HuCAL<sup>®</sup>-Fab 1 was cloned into a phagemid expression vector pMORPH<sup>®</sup>18\_Fab1 (figure 2). This vector comprises the Fd fragment with a phoA signal sequence fused at the C-terminus to a truncated gene III protein of filamentous phage, and further comprises the light chain VL-CL with an ompA signal sequence. Both chains are under the control of the lac operon. The constant domains C $\lambda$ , C $\kappa$  and CH1 are synthetic genes fully compatible with the modular system of HuCAL<sup>®</sup> (Knappik, (2000), *loc. cit.*).

The whole VH-chain (*MunI/StyI*-fragment) was replaced by a 1205 bp dummy fragment containing the  $\beta$ -lactamase transcription unit (*bla*), thereby facilitating subsequent steps for vector fragment preparation and allowing for selection of complete VH removal.

After VH-replacement, VL $\lambda$  was removed by *EcoRI/DraIII* and VL $\kappa$  by *EcoRI/BsiWI* and replaced with bacterial alkaline phosphatase (*bap*) gene fragment (1420 bp).

As the variability of the light chains is lower than that of the heavy chains, cloning was started with the light chain libraries. The VL $\lambda$  and VL $\kappa$  light chain libraries diversified in L-CDR3, which were generated for the HuCAL<sup>®</sup>-scFv library (Knappik, (2000), *loc. cit.*) were also used for cloning of HuCAL<sup>®</sup>-Fab1. In case of  $\lambda$  they consisted of the  $\lambda$ 1-,  $\lambda$ 2- and  $\lambda$ 3-HuCAL<sup>®</sup>-framework and had a total variability of  $5.7 \times 10^6$ . VL $\lambda$  fragments were amplified by 15 PCR cycles (Pwo-polymerase) with primers 5'-GTGGTGGTTCCGATATC-3' (SEQ ID NO: 28) and 5'-AGCGTCACACTCGGTGCGGCTTTTCGGCTGGCCAAGAACGGTTA-3' (SEQ ID NO: 29). PCR-products were digested with *EcoRV/DraIII* and gel-purified. In case of the VL $\lambda$ -library, the *bap*-dummy was removed by *EcoRV/DraIII* from the library vector. 2  $\mu$ g of gelpurified vector were ligated with a 3-fold molar excess of VL $\lambda$ -chains for 16 h at 16°C, and the ligation mixtures were electroporated in 800  $\mu$ l *E. coli* TOP10F cells (Invitrogen), yielding altogether  $4.1 \times 10^8$  independent colonies.

The transformants were amplified about 2000-fold in 2 x YT/1% glucose/34 µg/ml chloramphenicol/100 µg/ml ampicillin, harvested and stored in 20% (w/v) glycerol at -80°C.

The  $\kappa$  libraries comprise the  $\kappa 1$ -,  $\kappa 2$ -,  $\kappa 3$ - and  $\kappa 4$ -HuCAL<sup>®</sup> master genes with a total variability of  $5.7 \times 10^6$ . VL $\kappa$ -chains were obtained by restriction digest with *EcoRV/BsWI* and gel-purified. In case of the VL $\kappa$ -library, the *bap*-dummy was removed by *EcoRV/BsWI* from the library vector. 2 µg of gel-purified vector were mixed with a 5-fold molar excess of VL $\kappa$ -chains. Ligation and transformation into *E. coli* TOP10F cells (Invitrogen) was performed as described for VL $\lambda$ -chains, yielding altogether  $1.6 \times 10^8$  independent colonies.

DNA of the two light chain libraries was prepared and the *bla*-dummy was removed by *MunII/StyI*, thereby generating the two vectors for insertion of the VH sub-libraries. The VH libraries of HuCAL<sup>®</sup>-scFv were used for the generation of HuCAL<sup>®</sup>-Fab1. The VH libraries of HuCAL<sup>®</sup>-scFv consist of the master genes VH1A/B-6 diversified with two VH-CDR3 trinucleotide library cassettes differing in CDR3 length separately, and each VH-library combined with the VL $\kappa$ - and with the VL $\lambda$ -library. For the generation of the HuCAL<sup>®</sup>-Fab1 DNA from these VH-libraries was prepared preserving the original variability. The DNA was digested with *MunII/StyI* and gel-purified. A 5-fold molar excess of the VH-chains was ligated with 3 µg of the VL $\lambda$ -library vector and with 3 µg of the VL $\kappa$ -library vector for 4 h at 22°C. The ligation mixtures were electroporated for each vector in 1200 µl *E. coli* TOP10F cells (Invitrogen), yielding altogether  $2.1 \times 10^{10}$  independent colonies. The transformants were amplified about 4000-fold in 2 x YT/1% glucose/34 µg/ml chloramphenicol/10 µg/ml tetracycline, harvested and stored in 20% (w/v) glycerol at -80°C.

As quality control the light chain and heavy chain of single clones was sequenced with 5'-CAGGAAACAGCTATGAC-3' (SEQ ID NO: 30) and 5'-TACCGTTGCTCTTCACCCC-3' (SEQ ID NO: 31), respectively.

#### *Phagemid rescue, phage amplification and purification*

HuCAL<sup>®</sup>-Fab 1 was amplified in 2 x TY medium containing 34 µg/ml chloramphenicol, 10 µg/ml tetracycline and 1 % glucose (2 x TY-CG). After helper phage infection (VCSM13) at 37°C at an OD<sub>600</sub> of about 0.5, centrifugation and

resuspension in 2 x TY / 34 µg/ml chloramphenicol / 50 µg/ml kanamycin cells were grown overnight at 30°C. Phage were PEG-precipitated from the supernatant (Ausubel, (1998), Current protocols in molecular biology. John Wiley & Sons, Inc., New York, USA), resuspended in PBS/20% glycerol and stored at -80°C. Phage amplification between two panning rounds was conducted as follows: mid-log phase TG1-cells were infected with eluted phage and plated onto LB-agar supplemented with 1% of glucose and 34 µg/ml of chloramphenicol. After overnight incubation at 30°C colonies were scraped off, adjusted to an OD<sub>600</sub> of 0.5 and helper phage added as described above.

#### **Example 2: Solid phase panning**

Wells of MaxiSorp™ microtiterplates F96 (Nunc) were coated with 100 µl 2.5 µM human Aβ (1-40) peptide (Bachem) dissolved in TBS containing NaN<sub>3</sub> (0.05% v/v) and the sealed plate was incubated for 3 days at 37 °C where the peptide is prone to aggregate on the plate. After blocking with 5% non-fat dried milk in TBS, 1–5 x 10<sup>12</sup> HuCAL®-Fab phage purified as above were added for 1h at 20°C. After several washing steps, bound phages were eluted by pH-elution with 500 mM NaCl, 100 mM glycine pH 2.2 and subsequent neutralisation with 1M TRIS-Cl pH 7. Three rounds of panning were performed with phage amplification conducted between each round as described above, the washing stringency was increased from round to round.

#### **Example 3: Subcloning of selected Fab fragments for expression**

The Fab-encoding inserts of the selected HuCAL®-Fab fragments were subcloned into the expression vector pMORPH®x7\_FS to facilitate rapid expression of soluble Fab. The DNA preparation of the selected HuCAL®-Fab clones was digested with *XbaI/EcoRI*, thus cutting out the Fab encoding insert (ompA-VL and phoA-Fd). Subcloning of the purified inserts into the *XbaI/EcoRI* cut vector pMORPH®x7, previously carrying a scFv insert, leads to a Fab expression vector designated pMORPH®x9\_Fab1 (figure 3). Fabs expressed in this vector carry two C-terminal tags (FLAG and Strep) for detection and purification.

#### Example 4: Identification of A $\beta$ -binding Fab fragments by ELISA

Wells of Maxisorp™ microtiterplates F384 (Nunc) were coated with 20  $\mu$ l 2.5  $\mu$ M human A $\beta$  (1-40) peptide (Bachem) dissolved in TBS containing NaN<sub>3</sub> (0.05% v/v) and the sealed plate was incubated for 3 days at 37 °C, where the peptide is prone to aggregate on the plate. Expression of individual Fab was induced with 1 mM IPTG for 16 h at 22°C. Soluble Fab was extracted from *E. coli* by BEL lysis (boric acid, NaCl, EDTA and lysozyme containing buffer pH 8) and used in an ELISA. The Fab fragment was detected with an alkaline phosphatase-conjugated goat anti-Fab antibody (Dianova/Jackson Immuno Research). After excitation at 340 nm the emission at 535 nm was read out after addition of AttoPhos fluorescence substrate (Roche Diagnostics).

#### Example 5

*Optimization of antibody fragments.* In order to optimize the binding affinity of the selected A $\beta$  binding antibody fragments, some of the Fab fragments, MS-Roche-3 (MSR-3), MS-Roche-7 (MSR-7) and MS-Roche-8 (MSR-8) (figure 4), were used to construct a library of Fab antibody fragments by replacing the parental VL  $\kappa$ 3 chain by the pool of all kappa chains  $\kappa$ 1-3 diversified in CDR3 from the HuCAL® library (Knappik et al., 2000).

The Fab fragments MS-Roche-3, 7 and 8 were cloned via *Xba*I/*Eco*RI from pMORPH®x9\_FS into pMORPH®18, a phagemid-based vector for phage display of Fab fragments, to generate pMORPH®18\_Fab1 (figure 2). A kappa chain pool was cloned into pMORPH®18\_Fab1 via *Xba*I/*Sph*I restriction sites.

The resulting Fab optimization library was screened by panning against aggregated human A $\beta$  (1-40) peptide coated to a solid support as described in example 2.

Optimized clones were identified by koff-ranking in a Biacore assay as described in Example 7. The optimized clones MS-Roche-3.2, 3.3, 3.4, 3.6, 7.2, 7.3, 7.4, 7.9, 7.11, 7.12, 8.1, 8.2, were further characterized and showed improved affinity and

biological activity compared to the starting fragment MS-Roche-3, MS-Roche-7 and MS-Roche-8 (figure 4). The CDRs listed refer to the HuCAL<sup>®</sup> consensus-based antibody gene VH3kappa3. The Fab fragment MS-Roche-7.12 and its derivatives were obtained by cloning the HCDR3 library cassette out of the HuCAL<sup>®</sup>-Fab1 into a HuCAL<sup>®</sup>-library, carrying diversity in all 6 CDR regions as described in Knappik (Knappik *et al.*, 2000), by mimicking the natural distribution of amino acid residues at every CDR position, and by taking canonical structures and residues into account appropriately (Allazikani *et al.*, 1997). However in contrast to the HuCAL<sup>®</sup> master genes, the clone MS-Roche 7.12 contains amino acid S at position 49 of the VL chain (see appended table 1).

The optimized Fabs after the first affinity maturation round showed improved characteristics over the starting MS-Roche-3, MS-Roche-7 and MS-Roche-8 clones (Figure 4). The binding affinities of the matured Fabs to A $\beta$ 1-40 and A $\beta$ 1-42 were significantly increased yielding  $K_D$  values in the range of 22 – 240 nM in comparison to 850 – 1714 nM of the parental clones (Table 3). Immunohistochemistry analysis of amyloid plaques in human AD brain sections also showed a significantly increased staining profile of the matured clones, i. e. better signal to background ratios were obtained and positive plaque staining was detected at relatively low concentrations of the matured Fabs (Figure 5).

For further optimization, the VH CDR2 regions and the VL CDR1 regions of a set of antibody fragments derived from L-CDR3 optimized MS-Roche-3, -7 and -8 (table 1; figure 4) were optimized by cassette mutagenesis using trinucleotide-directed mutagenesis (Virnekäs *et al.*, 1994). Therefore, a trinucleotide-based HCDR2 cassette and a trinucleotide-based LCDR1 cassette were constructed using a design procedure identical with that for CDR3 cassettes described in Knappik *et al.*, 2000. The library cassettes were designed strongly biased for the known natural distribution of amino acids and following the concept of canonical CDR conformations established by Allazikani (Allazikani *et al.*, 1997). The protocol used for the optimization of the initial selected antibody fragments would mimic the process of affinity maturation by somatic hypermutation observed during the natural immune response.

The resulting libraries were screened separately as described above leading to optimized clones either in the H-CDR2 or in the L-CDR1 region. All clones were identified as above by an improved koff towards A $\beta$ 1-40-fibers after a koff-ranking in the Biacore and showed improved affinity either to A $\beta$ 1-40 or A $\beta$ -42 or both when compared to the corresponding parent clone (Table 3). Table 1 contains the sequence characteristics of the parental as well as sequences of the optimized clones. The CDRs listed refer to the HuCAL<sup>®</sup> consensus-based antibody gene VH3kappa3.

For example, the affinity of the MS-Roche-7 parental Fab towards Ab1-40 was improved over 35-fold from 1100 nM to 31 nM after L-CDR3 optimization (MS-Roche-7.9) and further improved to 5 nM after H-CDR2 optimization (MS-Roche-7.9H2) as illustrated in Table 3.

The H-CDR2 and L-CDR1 optimization procedure not only increased the affinity but also resulted for some of the clones in a significantly improved staining of amyloid plaques in AD brain section, as particularly seen with MS-Roche 7.9H2 and 7.9H3.



Table 1

Binder name	L-CDR1	pos. 49	L-CDR2	pos. 85	L-CDR3	H-CDR1	pos. 47	H-CDR2	H-CDR3
MS-Roche #3	RASQSVSSSYLA	Y	GASSRAT	V	QQVNNPPV	GTFSSYAMS	W	AISGSGGSTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.1	RASQSVSSSYLA	Y	GASSRAT	T	QQVSVPPP	GTFSSYAMS	W	AISGSGGSTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.2	RASQSVSSSYLA	Y	GASSRAT	V	QQIYSYPP	GTFSSYAMS	W	AISGSGGSTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.3	RASQSVSSSYLA	Y	GASSRAT	V	HQMSSYPP	GTFSSYAMS	W	AISGSGGSTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISGSGGSTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.5	RASQSVSSSYLA	Y	GASSRAT	T	QQIYDYPP	GTFSSYAMS	W	AISGSGGSTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.6	RASQSVSSSYLA	Y	GASSRAT	V	QQTYNYPP	GTFSSYAMS	W	AISGSGGSTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.2.H1	RASQSVSSSYLA	Y	GASSRAT	V	QQIYSYPP	GTFSSYAMS	W	AISEHGLNIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.2.H2	RASQSVSSSYLA	Y	GASSRAT	V	QQIYSYPP	GTFSSYAMS	W	AISQRGQFTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.3.H1	RASQSVSSSYLA	Y	GASSRAT	V	HQMSSYPP	GTFSSYAMS	W	WISEKSRFIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.3.H2	RASQSVSSSYLA	Y	GASSRAT	V	HQMSSYPP	GTFSSYAMS	W	VISQESQKYIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.3.H3	RASQSVSSSYLA	Y	GASSRAT	V	HQMSSYPP	GTFSSYAMS	W	AISQNGFHIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H1	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISETSIRKYIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H2	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	VIDMVGHYYIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H3	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	VISQTKRKIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H4	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISETGMHIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H5	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	VISQVGAHIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H6	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISESGWSTYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H7	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	VISETGKNIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H8	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISEHGRFKYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H9	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISESSKNKYIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H10	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISESGRGKYIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H11	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISEFGKNIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H12	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	VISQIQGNIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H13	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISEQQGRNIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H14	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISESGQKYIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H16	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISESGVNIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H17	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISEFGQFIYYADSVKVG	LTHYARYRYFDV
MS-Roche #3.4.H18	RASQSVSSSYLA	Y	GASSRAT	T	QQTYDYPP	GTFSSYAMS	W	AISQQSNFIYYADSVKVG	LTHYARYRYFDV

MS-Roche #3.4.L7	RASQRLGRLYLA	Y	GASSRAT	T	QQTYYDPP	GTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYRYFDV
MS-Roche #3.4.L8	RASQWTKSYLA	Y	GASSRAT	T	QQTYYDPP	GTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYRYFDV
MS-Roche #3.4.L9	RASRRHVVYLA	Y	GASSRAT	T	QQTYYDPP	GTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYRYFDV
MS-Roche #3.4.L11	RASQLVGRAYLA	Y	GASSRAT	T	QQTYYDPP	GTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.H1	RASQSVSSSYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	VISEGQYKYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.H2	RASQSVSSSYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	VISERGINTYYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.H3	RASQSVSSSYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	VISETGFIYYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.H4	RASQSVSSSYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	AISERGRHIYYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.H5	RASQSVSSSYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	AISESGTKYYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.H6	RASQSVSSSYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	AISEHGTNIYYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.H8	RASQSVSSSYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	AISEYSKFKYYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.L1	RASQFIQRFYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYRYFDV
MS-Roche #3.6.L2	RASQFLSRYLA	Y	GASSRAT	V	QQTYYNPP	GTFSSYAMS	W	AISGSGGSTYYADSVKG	LTHYARYRYFDV
MS-Roche #7	RASQSVSSSYLA	Y	GASSRAT	T	FQLYSDPF	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.1	RASQSVSSSYLA	Y	GASSRAT	V	HQLYSSPY	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.2	RASQSVSSSYLA	Y	GASSRAT	T	QQIYSFPH	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.3	RASQSVSSSYLA	Y	GASSRAT	V	HQVYSHPF	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.4	RASQSVSSSYLA	Y	GASSRAT	V	QQIYNFPH	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.5	RASQSVSSSYLA	Y	GASSRAT	T	HQVYSSPF	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.6	RASQSVSSSYLA	Y	GASSRAT	V	HQLYSPPY	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.7	RASQSVSSSYLA	Y	GASSRAT	T	HQVYSAPF	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.8	RASQSVSSSYLA	Y	GASSRAT	V	HQVYSFPI	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.9	RASQSVSSSYLA	Y	GASSRAT	T	LQIYNMPI	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.10	RASQSVSSSYLA	Y	GASSRAT	T	QQVYNPPH	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF
MS-Roche #7.11	RASQSVSSSYLA	Y	GASSRAT	T	QQVYSPPH	GTFSSYAMS	W	AISGSGGSTYYADSVKG	GKGNTHKPYGVWRYF

MS-Roche #7.12	RASQVVSPPYLA	S	GSSNRAT	V	LQLYNIPN	GFTFSSYGMS	W	NISGSGSSTYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.13	RASQSVSSSYLA	Y	GASSRAT	V	HQVYSPPF	GFTFSSYAMS	W	AIISGSGGSTYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.2.H1	RASQSVSSSYLA	Y	GASSRAT	T	QQIYSFPH	GFTFSSYAMS	W	AINANGLKYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.2.H2	RASQSVSSSYLA	Y	GASSRAT	T	QQIYSFPH	GFTFSSYAMS	W	AINGTGMKKYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.2.H3	RASQSVSSSYLA	Y	GASSRAT	T	QQIYSFPH	GFTFSSYAMS	W	AINANGYKTYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.2.H4	RASQSVSSSYLA	Y	GASSRAT	T	QQIYSFPH	GFTFSSYAMS	W	AINSKGSRIYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.2.H5	RASQSVSSSYLA	Y	GASSRAT	T	QQIYSFPH	GFTFSSYAMS	W	AINATGRSKYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.2.H7	RASQSVSSSYLA	Y	GASSRAT	T	QQIYSFPH	GFTFSSYAMS	W	AINSRGSDTHYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.2.L1	RASQYVDRTYLA	Y	GASSRAT	T	QQIYSFPH	GFTFSSYAMS	W	AIISGSGGSTYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.3.H1	RASQSVSSSYLA	Y	GASSRAT	V	HQVYSHPF	GFTFSSYAMS	W	AIASISNKTYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.4.H1	RASQSVSSSYLA	Y	GASSRAT	V	QQIYNFPH	GFTFSSYAMS	W	AINATGYRTYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.4.H2	RASQSVSSSYLA	Y	GASSRAT	V	QQIYNFPH	GFTFSSYAMS	W	AINYNGARIYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.9.H1	RASQSVSSSYLA	Y	GASSRAT	T	LQIYNMPI	GFTFSSYAMS	W	AINANGQRKFYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.9.H2	RASQSVSSSYLA	Y	GASSRAT	T	LQIYNMPI	GFTFSSYAMS	W	AINADGNRKYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.9.H3	RASQSVSSSYLA	Y	GASSRAT	T	LQIYNMPI	GFTFSSYAMS	W	AINYQGNRKYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.9.H4	RASQSVSSSYLA	Y	GASSRAT	T	LQIYNMPI	GFTFSSYAMS	W	AINAVGMKKFYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.11.H1	RASQSVSSSYLA	Y	GASSRAT	T	QQVYSPPH	GFTFSSYAMS	W	GINAAGFRTYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.11.H2	RASQSVSSSYLA	Y	GASSRAT	T	QQVYSPPH	GFTFSSYAMS	W	AINANGYKYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.11.H3	RASQSVSSSYLA	Y	GASSRAT	T	QQVYSPPH	GFTFSSYAMS	W	GINANGNRITYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.11.H5	RASQSVSSSYLA	Y	GASSRAT	T	QQVYSPPH	GFTFSSYAMS	W	AINAHGQRTYYADSVKG	GKGNTHKPYGVVRYF DV
MS-Roche #7.12.L1	RASQYVFRYYLA	S	GSSNRAT	V	LQLYNIPN	GFTFSSYGMS	W	NISGSGSSTYYADSVKG	GKGNTHKPYGVVRYF DV

MS-Roche #7.12.L2	RASQRFFKYLA	S	GSSNRAT	V	LQLYNIPN	GFTFSSYGMS	W	NISGSGSSTYYADSVKG	DV	GKGNTHKPYGYVRYF
MS-Roche #7.12.L4	RASQRLKRSYLA	S	GSSNRAT	V	LQLYNIPN	GFTFSSYGMS	W	NISGSGSSTYYADSVKG	DV	GKGNTHKPYGYVRYF
MS-Roche #8	RASQSVSSSYLA	Y	GASSRAT	T	QQLSSFPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LLSRGYNGYYHKFDV	LLSRGYNGYYHKFDV
MS-Roche #8.1	RASQSVSSSYLA	Y	GASSRAT	T	QQLSNYPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LLSRGYNGYYHKFDV	LLSRGYNGYYHKFDV
MS-Roche #8.2	RASQSVSSSYLA	Y	GASSRAT	T	QQLSSYPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LLSRGYNGYYHKFDV	LLSRGYNGYYHKFDV
MS-Roche #8.1.H1	RASQSVSSSYLA	Y	GASSRAT	T	QQLSNYPP	GFTFSSYAMS	W	AISRSGSNYYADSVKG	LLSRGYNGYYHKFDV	LLSRGYNGYYHKFDV
MS-Roche #8.2.H1	RASQSVSSSYLA	Y	GASSRAT	T	QQLSSYPP	GFTFSSYAMS	W	AISITGRRKYYADSVKG	LLSRGYNGYYHKFDV	LLSRGYNGYYHKFDV
MS-Roche #8.2.H2	RASQSVSSSYLA	Y	GASSRAT	T	QQLSSYPP	GFTFSSYAMS	W	AISRTGSKTYADSVKG	LLSRGYNGYYHKFDV	LLSRGYNGYYHKFDV
MS-Roche #8.2.H4	RASQSVSSSYLA	Y	GASSRAT	T	QQLSSYPP	GFTFSSYAMS	W	ATSVKGTKTYADSVKG	LLSRGYNGYYHKFDV	LLSRGYNGYYHKFDV
MS-Roche #8.2.L1	RASQRVSGRYLA	Y	GASSRAT	T	QQLSSYPP	GFTFSSYAMS	W	AISGSGGSTYYADSVKG	LLSRGYNGYYHKFDV	LLSRGYNGYYHKFDV

Sequences in "black" HuCAL-consensus

## Example 6

### *Construction of HuCAL<sup>®</sup> immunoglobulin expression vectors*

**Heavy chain cloning.** The multiple cloning site of pCDNA3.1+ (Invitrogen) was removed (*NheI/ApaI*), and a stuffer compatible with the restriction sites used for HuCAL<sup>®</sup> design was inserted for the ligation of the leader sequences (*NheI/EcoRI*), VH-domains (*MunI*), and the immunoglobulin constant regions (*BspI/ApaI*). The leader sequence (EMBL 83133) was equipped with a Kozak sequence (Kozak, 1987). The constant regions of human IgG (PIR J00228), IgG4 (EMBL K01316), and serum IgA1 (EMBL J00220) were dissected into overlapping oligonucleotides with length of about 70 bases. Silent mutations were introduced to remove restriction sites non-compatible with the HuCAL<sup>®</sup> design. The oligonucleotides were spliced by overlap extension-PCR.

**Light chain cloning.** The multiple cloning site of pCDNA3.1/Zeo+ (Invitrogen) was replaced by two different stuffers. The  $\kappa$ -stuffer provided restriction sites for insertion of a  $\kappa$ -leader (*NheI/EcoRV*), HuCAL<sup>®</sup>-scFv V $\kappa$ -domains (*EcoRV/BsiWI*), and the  $\kappa$ -chain constant region (*BsiWI/ApaI*). The corresponding restriction sites in the  $\lambda$ -stuffer were *NheI/EcoRV* ( $\lambda$ -leader), *EcoRV/HpaI* (V $\lambda$ -domains), and *HpaI/ApaI* ( $\lambda$ -chain constant region). The  $\kappa$ -leader (EMBL Z00022) as well as the  $\lambda$ -leader (EMBL J00241) were both equipped with Kozak sequences. The constant regions of the human  $\kappa$ - (EMBL L00241) and  $\lambda$ -chain (EMBL M18645) were assembled by overlap extension-PCR as described above.

**Generation of IgG-expressing CHO-cells.** CHO-K1 cells were co-transfected with an equimolar mixture of IgG heavy and light chain expression vectors. Double-resistant transfectants were selected with 600  $\mu$ g/ml G418 and 300  $\mu$ g/ml Zeocin (Invitrogen) followed by limiting dilution. The supernatant of single clones was assessed for IgG expression by capture-ELISA. Positive clones were expanded in RPMI-1640 medium supplemented with 10% ultra-low IgG-FCS (Life Technologies). After adjusting the pH of the supernatant to 8.0 and sterile filtration, the solution was subjected to standard protein A column chromatography (Poros 20 A, PE Biosystems).

### Example 7: Pepspot analysis with decapeptides

The following aminoacid sequence encompassing A $\beta$  (1-42) was divided into 43 overlapping decapeptides with a frameshift of 1 aminoacid.

ISEVKM<sup>1</sup>DAEF RHDSGYEVHH QKLVFFAEDV GSNKGAIIGL MVGGVVI<sup>42</sup>ATV IV

The 43 decapeptides were synthesized with N-terminal acetylation and C-terminal covalent attachment to a cellulose sheet ("pepspot") by a commercial supplier (Jerini BioTools, Berlin). The cellulose sheet is incubated for 2 hours on a rocking platform with monoclonal antibody (2 ug/ml) in blocking buffer (50 mM Tris·HCl, 140 mM NaCl, 5 mM NaEDTA, 0.05% NP40 (Fluka), 0.25% gelatine (Sigma), 1% bovine serum albumine fraction V (Sigma), pH 7.4). The sheet is washed 3 times 3 minutes on a rocking platform with TBS (10 mM Tris·HCl, 150 mM NaCl, pH 7.5). It is then wetted with Kathode buffer (25 mM Tris base, 40 mM 6-Aminohexane acid, 0.01% SDS, 20% Methanol) and transferred to a semi-dry blotting stack with the peptide side facing a PVDF membrane (Biorad) of equal size.

The semi-dry blotting stack consists out of freshly wetted filter papers (Whatman No.3) slightly larger than the peptide sheet:

3 papers wetted with Cathode buffer

the peptide sheet

a sheet of PVDF membrane wetted with methanol

3 papers wetted with Anode buffer 1 (30mM Tris base, 20% methanol)

3 papers wetted with Anode buffer 2 (0.3 mM Tris base, 20% methanol)

The transfer is conducted at a current density between Cathode and Anode of 0.8 mA/cm<sup>2</sup> for 40 minutes which is sufficient to elute most of the antibody from the cellulose sheet and deposit it on the PVDF membrane. The PVDF membrane is then exchanged for a 2<sup>nd</sup> PVDF membrane and transferred for another 40 minutes to ensure complete elution from the cellulose sheet.

The PVDF membrane is immersed in blocking buffer for 10 minutes. Then HRP-labeled anti-human Ig H+L (Pierce) is added at 1:1000 dilution and the membrane is incubated on a rocking platform for 1 hour. It is washed 3x10 minutes with TBST (TBS with 0.005% Tween20). Color is developed by immersing the membrane into a solution made of 3mg 4-chloronaphtol dissolved in 9 ml methanol with 41 ml PBS (20 mM Na-phosphate, 150 mM NaCl, pH 7.2) and 10 ul 30% hydrogen peroxide

(Merck). After the development of blue-black spots the membrane is washed extensively with water and dried.

The assignment of antibody-reactive pepspots is made by visual inspection through a transparent spot matrix. The epitopes of the antibody in question is defined as the minimal aminoacid sequence in reactive peptides. For comparison mouse monoclonal antibodies (BAP-2, BAP-21, BAP-24, and 4G8) are analyzed in the same way, except using HRP-labeled anti-mouse Ig instead of anti-human Ig.

antibody	position	position
MSR-3	3-4	18-23
MSR-7	3-5	19-24
MSR-8	4-5	18-21
MSR-9	(1)3-9	18-24
MSR-10	(4-10)	19-20
MSR-11	3-7	(18-20)
MSR-26	3-5	(16)-19-23
MSR-27	(3)6-9	13-18(20)
MSR-29		14-16(20)
MSR-37	(4-6)	(19-24)
MSR-41	3-7	(17-21)
MSR-42	(4-9)	(18-24)
BAP-2	4-6	
4G8		19-20(23)
BAP-21		32-34
BAP-24		38-40

Table 2: Pepspot analysis with decapeptides. The minimal epitopes are given as the amino acid position in the A $\beta$  1-40 sequence. A weak peptide reactivity, and hence a weak contribution to the epitope, is indicated by brackets.

**Example 8: Determination of  $K_D$  values for MS-R Fab and MS-R IgG1 antibody binding to A $\beta$ 1-40 and A $\beta$ 1-42 fibers *in vitro* by surface plasmon resonance (SPR)**

Binding of anti-A $\beta$  antibodies (Fabs and IgG1) to fibrillar A $\beta$  was measured online by surface plasmon resonance (SPR), and the affinities of the molecular interactions were determined as described by Johnson, *Anal. Biochem.* 1991, 198, 268 – 277, and Richalet-Sécordel, *Anal. Biochem.* 1997, 249, 165 – 173. Biacore2000 and Biacore3000 instruments were used for these measurements. A $\beta$ 1-40 and A $\beta$ 1-42 fibers were generated *in vitro* by incubation of synthetic peptides at a concentration of 200  $\mu$ g/ml in 10 mM Na-acetat buffer (pH 4.0) for three days at 37°C. Electron microscopic analysis confirmed a fibrillar structure for both peptides, A $\beta$ 1-40 showing predominantly shorter (< 1 micron) and A $\beta$ 1-42 predominantly longer (> 1 micron) fibers. These fibers are assumed to represent aggregated A $\beta$  peptides in human AD brain more closely than ill-defined mixtures of amorphous aggregates and unstructured precipitates. The fibers were diluted 1:10 and directly coupled to a "Pioneer Sensor Chip F1" as described in the Instruction Manual of the manufacturer (BIAapplication Handbook, version AB, Biacore AB, Uppsala, 1998). In initial experiments it was found that selected MS-Roche Fabs differed substantially in their reaction kinetics and therefore the mode of data analysis had to be chosen accordingly. For binders with slow kinetics  $K_D$  values were calculated by curve fitting of the time-dependent sensor responses, i. e. from the ratio of  $k_{off}/k_{on}$ . Binders with fast kinetics were analyzed by fitting the concentration-dependent sensor responses at equilibrium (adsorption-isotherms).  $K_D$  values were calculated from the Biacore sensograms based on the total Fab concentration as determined by a protein assay. For the clones derived from the 1<sup>st</sup> and 2<sup>nd</sup> affinity maturation cycle the content of active Fab in each preparation was determined in the Biacore according to a method described by Christensen, *Analytical Biochemistry* (1997) 249, 153 –164. Briefly, time-dependent protein binding to A $\beta$ 1-40 fibers immobilized on the Biacore chip was measured during the association phase under mass-limited conditions at different flow rates of the analyte solution. The conditions of mass limitation were realized by immobilizing high amounts of A $\beta$  fibers (2300 response units) on the chip



surface of a measuring channel and by working at relatively low analyte concentrations, i. e. 160 nM (based on the total Fab protein concentration).

A summary of the  $K_D$  values of selected MS-Roche clones identified in the primary screen of the HuCAL library and their corresponding matured derivatives after the 1<sup>st</sup> and 2<sup>nd</sup> affinity maturation cycle is shown in Table 3. In the 1<sup>st</sup> affinity maturation cycle the heavy chain CDR3 (VH-CDR3) was kept constant and optimization was focussed on randomization of the light chain CDR3 (VL-CDR3). In the 2<sup>nd</sup> affinity cycle randomization of VL-CDR1 and VH-CDR2 was performed. Some of the binders from the 1<sup>st</sup> maturation cycle were converted to full-length human IgG1 antibodies according to the technology developed by MorphoSys as described in Example 6 and  $K_D$  values determined in the Biacore as described above. The  $K_D$  values for full-length IgG1 binding to A $\beta$ 1-40 and A $\beta$ 1-42 fibers are shown in Table 4.

Secreted clones from	MS-R #	$K_D$ $A\beta_{1-40}$ nM	$K_D$ $A\beta_{1-42}$ nM	MS-R #	$K_D$ $A\beta_{1-40}$ nM	$K_D$ $A\beta_{1-42}$ nM	MS-R #	$K_D$ $A\beta_{1-40}$ nM	$K_D$ $A\beta_{1-42}$ nM
primary screen	3	930	1300	7	1100	1714	8	850	1000
1 <sup>st</sup> affinity maturation	3.2	52	240	7.2	22	58	8.1	24	42
	3.3	38	104	7.3	23	88	8.2	24	64
	3.4	32	103	7.4	28	103			
	3.6	40	68	7.9	31	93			
				7.11	22	74			
				7.12	28	60			
2 <sup>nd</sup> affinity maturation	3.2H1	4.4	3.3	7.2H1	9.3	10.2	8.1H1	13.6	9.2
	3.2H2	5.2	1.1	7.2H2	8.2	8.2	8.2H1	1.6 <sup>a</sup>	2.1 <sup>a</sup>
	3.3H1	17.1	19.4	7.2H3	45.4	5.3	8.2H3	n.d.	3.1
	3.3H2	10.6	22.8	7.2H4	5.9	5.0	8.2H4	12.1	11.9
	3.3H3	1.4	3.3	7.2H5	8.0	10.1	8.2L1	4.8	3.7
	3.4H1	13.5	14.0	7.2H7	15.5	8.1			
	3.4H3	6.7	8.4	7.2L1	13.3	12.7			
	3.4H4	33.0	43.0	7.3H1	8.0	11.2			
	3.4H5	26.5	36.0	7.4H1	8.0	6.6			
	3.4H6	49.0	60.0	7.4H2	9.9	6.2			
	3.4H7	19.2	31.7	7.9H1	4.9	5.4			
	3.4H8	10.7	26.5	7.9H2	5.0	5.7			
	3.4H9	21.7	18.6	7.9H3	4.2	2.8			
	3.4H10	8.1	10.1	7.9H4	4.8	4.2			
	3.4H11	19.5	8.3	7.11H1	12.7	6.7			
	3.4H12	25.5	27.0	7.11H2	0.3	0.3			
	3.4H13	32.3	18.8	7.11H3	6.6	4.4			
	3.4H14	13.3	16.8	7.11H5	3.4	1.7			
	3.4H16	25.5	15.6	7.12L1	n.d.	3.8			
	3.4H17	2.0	4.3	7.12L2	4.0	5.4			
	3.4H18	17.1	10.0	7.12L4	2.0	0.6			
	3.4L7	9.3	9.3						
	3.4L8	6.2	13.0						
	3.4L9	16.3	9.1						
	3.4L11	5.3	2.6						
	3.6H1	18.9	23.1						
	3.6H2	19.8	54.0						
	3.6H3	5.4	7.5						
	3.6H4	13.0	7.8						
	3.6H5	8.2	6.0						
	3.6H6	36.0	11.8						
	3.6H8	2.5	2.5						
	3.6L1	15.6	11.1						
	3.6L2	13.7	13.1						

**Table 3:**  $K_D$  values for MS-R Fab binding to  $A\beta_{1-40}$  and  $A\beta_{1-42}$  fibers as determined in the Biacore. For the clones derived from the 1<sup>st</sup> and 2<sup>nd</sup> affinity maturation cycle the values are corrected for the content of active Fab present in each sample as described in the text. <sup>a</sup>, values were calculated from the concentration-dependent sensor responses at equilibrium; n.d., not determined.

MS-R #	$K_D$ A $\beta$ <sub>1-40</sub> nM	$K_D$ A $\beta$ <sub>1-42</sub> nM
3.3 IgG1	3.7	6.6
7.11 IgG1	2.3	5.7
7.12 IgG1	3.1	13.7
8.1 IgG1	6.6	12.3

**Table 4:**  $K_D$  values for MS-R IgG1 binding to A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> fibers as determined in the Biacore. The IgGs were derived from MS-R Fabs selected after the 1<sup>st</sup> affinity maturation cycle. The values are corrected for the content of active MS-R IgGs present in each sample as described in the text.

**Example 9: Staining of genuine human amyloid plaques in brain sections of an Alzheimer's Disease patient by indirect immunofluorescence**

Selected MS-Roche Fabs and full-length IgG1 were tested for binding to  $\beta$ -amyloid plaques by immunohistochemistry analysis. Cryostat sections of unfixed tissue from human temporal cortex (obtained postmortem from a patient that was positively diagnosed for Alzheimer's disease) were labeled by indirect immunofluorescence using MS-Roche Fabs or corresponding full-length human IgG1 antibodies at various concentrations. Fabs and IgG1 antibodies were revealed by goat anti-human affinity-purified F(ab')<sub>2</sub> fragment conjugated to Cy3 and goat anti-human (H+L) conjugated to Cy3, respectively. Both secondary reagents were obtained from Jackson Immuno Research. Controls included an unrelated Fab and the secondary antibodies alone, which all gave negative results. Typical examples of plaque stainings with selected MS-Roche Fabs and MS-Roche IgG1 antibodies are shown in Figures 5 and 6.

**Example 10: Polymerization Assay: Prevention of A $\beta$  aggregation**

Synthetic A $\beta$  when incubated in aqueous buffer over several days spontaneously aggregates and forms fibrillar structures which are similar to those seen in amyloid deposits in the brains of Alzheimer's Disease patients. We have developed an *in vitro* assay to measure incorporation of biotinylated A $\beta$  into preformed A $\beta$  aggregates in order to analyze the A $\beta$ -neutralizing potential of anti-A $\beta$  antibodies and other A $\beta$ -binding proteins such as albumin (Bohrmann et al., 1999, J. Biol. Chem. 274, 15990-

15995). The effect of small molecules on A $\beta$  aggregation can also be analyzed in this assay.

*Experimental procedure:*

NUNC Maxisorb microtiter plates (MTP) are coated with a 1:1 mixture of A $\beta$ 1-40 and A $\beta$ 1-42 (2  $\mu$ M each, 100  $\mu$ l per well) at 37°C for three days. Under these conditions highly aggregated, fibrillar A $\beta$  is adsorbed and immobilized on the surface of the well. The coating solution is then removed and the plates are dried at room temperature for 2-4 hours. (The dried plates can be stored at -20°C). Residual binding sites are blocked by adding 300  $\mu$ l/well phosphate-buffered saline containing 0.05 % Tween 20 (T-PBS) and 1 % bovine serum albumin (BSA). After 1-2 hours incubation at room temperature the plates are washed 1 x with 300  $\mu$ l T-PBS. A solution of 20 nM biotinylated A $\beta$ 1-40 in 20 mM Tris-HCl, 150 mM NaCl pH 7.2 (TBS) containing 0.05 % NaN<sub>3</sub> and serially diluted antibody is added (100  $\mu$ l/well) and the plate incubated at 37°C overnight. After washing 3 x with 300  $\mu$ l T-PBS a streptavidin-POD conjugate (Roche Molecular Biochemicals), diluted 1:1000 in T-PBS containing 1% BSA, is added (100  $\mu$ l/well) and incubated at room temperature for 2 hours. The wells are washed 3 x with T-PBS and 100  $\mu$ l/well of a freshly prepared tetramethyl-benzidine (TMB) solution are added. [Preparation of the TMB solution: 10 ml 30 mM citric acid pH 4.1 (adjusted with KOH) + 0.5 ml TMB (12 mg TMB in 1 ml acetone + 9 ml methanol) + 0.01 ml 35 % H<sub>2</sub>O<sub>2</sub>]. The reaction is stopped by adding 100  $\mu$ l/well 1 N H<sub>2</sub>SO<sub>4</sub> and absorbance is read at 450 nm in a microtiter plate reader.

*Result:*

Figure 7 shows that MS-Roche IgG1 antibodies prevented incorporation of biotinylated A $\beta$ 1-40 into preformed A $\beta$ 1-40/A $\beta$ 1-42 aggregates. The A $\beta$ -neutralizing capacity of these full-length human IgGs was similar to that of the mouse monoclonal antibody BAP-1 which had been generated by a standard immunization procedure and specifically recognizes amino acid residues 4-6 of the A $\beta$ peptide when analyzed by the Pepspot technique as described in example 7. Mouse monoclonal antibody BAP-2 which also reacts exclusively with amino acids 4-6

(Brockhaus, unpublished) was significantly less active in this assay. An even lower activity was found with the A $\beta$ 1-40 C-terminal specific antibody BAP-17 (Brockhaus, Neuroreport 9 (1998), 1481-1486) and the monoclonal antibody 4G8 which recognizes an epitope between position 17 and 24 in the A $\beta$  sequence (Kim, 1988, Neuroscience Research Communication Vol. 2, 121-130). BSA at a concentration of up to 10  $\mu$ g/ml did not affect incorporation of biotinylated A $\beta$  and served as a negative control. However, at higher concentrations, i. e. > 100  $\mu$ g/ml, BSA has been reported to inhibit binding of biotinylated A $\beta$  into preformed A $\beta$  fibers (Bohrmann, (1999) *J Biol Chem* 274 (23), 15990-5) indicating that the interaction of BSA with A $\beta$  is not of high affinity.

#### **Example 11: De-polymerization Assay: Release of biotinylated A $\beta$ from aggregated A $\beta$**

In a similar experimental setup we have tested the potential of MS-Roche IgG antibodies to induce depolymerization of aggregated A $\beta$ . Biotinylated A $\beta$ 1-40 was first incorporated into preformed A $\beta$ 1-40/A $\beta$ 1-42 fibers before treatment with various anti-A $\beta$  antibodies. Liberation of biotinylated A $\beta$  was measured using the same assay as described in the polymerization assay.

##### ***Experimental procedure:***

NUNC Maxisorb microtiter plates (MTP) are coated with a 1:1 mixture of A $\beta$ 1-40 and A $\beta$ 1-42 as described in the polymerization assay. For incorporation of biotinylated A $\beta$  the coated plates are incubated with 200  $\mu$ l/well 20 nM biotinylated A $\beta$ 1-40 in TBS containing 0.05 % NaN<sub>3</sub> at 37°C overnight. After washing the plate with 3 x 300  $\mu$ l/well T-PBS, antibodies serially diluted in TBS containing 0.05 % NaN<sub>3</sub> were added and incubated at 37°C for 3 hours. The plate was washed and analyzed for the presence of biotinylated A $\beta$ 1-40 as described above.

##### ***Result:***

Figure 8 shows that antibodies of the HuCAL library induced de-polymerization of aggregated A $\beta$  as measured by the release of incorporated biotinylated A $\beta$ 1-40. The

MS-R antibodies and the mouse monoclonal antibody BAP-1 were similarly active whereas the BAP-2, BAP-17 and 4G8 antibodies were clearly less efficient in liberating biotinylated A $\beta$  from the bulk of immobilized A $\beta$  aggregates. It is interesting to note that BAP-2, despite its specificity for amino acid residue 4-6 which is exposed in aggregated A $\beta$  has a clearly lower activity in this assay indicating that not all N-terminus specific antibodies a priori are equally efficient in releasing A $\beta$  from preformed aggregates. The MS-Roche IgGs are clearly superior to BAP-2 with respect to the depolymerizing activity. The relatively low efficiency of BAP-17 (C-terminus-specific) and 4G8 (amino acid residues 16-24-specific) in this assay is due to the cryptic nature of these two epitopes in aggregated A $\beta$ . As already noted in the polymerization assay, BSA at the concentrations used here had no effect on aggregated A $\beta$ .

**EXAMPLE 12: Epitope analysis by ELISA on peptide conjugates.**


The following heptapeptides (single letter code) were obtained by solid-phase synthesis and purified by liquid chromatography using the techniques known in the art.

AEFRHDC  
EFRHDSC  
FRHDSGC  
RHDSGYC  
HDSGYEC  
DSGYEVC  
SGYEVHC  
YEVHHQC  
EVHHQKC  
VHHQKLC  
HHQKLVC  
HQKLVFC  
QKLVFFC  
KLVFFAC  
LVFFAEC  
VFFAEDC  
FFAEDVC  
FAEDVGC  
AEDVGSC  
EDVGSNC  
DVGSNKC

VGSNKGCC  
GSNKGAC  
CSNKGAI  
CNKGAIL  
CKGAILG  
CGLMVGG  
CMVGGVV  
CGGVVIA

The peptides were dissolved in DMSO to arrive at 10 mM concentration.

D Bovine Albumin (essentially fatty acid free BSA , Sigma Lot 112F-9390) was dissolved to 10 mg/ml in 0.1M sodium bicarbonate and activated by addition per ml of 50 ul of a 26 mg/ml solution of N-succinimidyl-maleinimido propionate (NSMP, Pierce) in DMSO. After 15 minutes reaction at room temperature the activated BSA was purified by gel filtration (NAP-10, Pharmacia) in PBS with 0.1% sodium azide as solvent. 50 ul of NSMP activated BSA ( 6.7 mg/ml) was diluted with 50 ul of PBS, 0.1% sodium azide and 10 ul of peptide solution (1 mM in DMSO) was added. As negative control activated BSA was mock-treated without peptide addition. After 4 hrs at room temperature the reaction was stopped by addition of 10 ul of 10mM Cystein. An aliquot of the conjugate reaction mixture was diluted 1:100 with 0.1M sodium bicarbonate buffer and immediately filled into the wells (100 ul) of ELISA plates (Nunc Immuno-Plate). After standing 16 hrs at 4°C 100 ul blocking buffer (as above) was added to each well and incubated for another 30 minutes. The plates were washed with 2x300ul/well TBST (as above) and filled with 100 ul antibody at 10 ug/ml or 2 µg/ml in blocking buffer. The plates were kept 16 hours at 4°C and washed with 2x300ul TBST. 100 ul/well HRP-conjugated anti-human Ig H+L (Pierce, dilution 1:1000 with blocking buffer) was added and incubated for 1 hour at ambient temperature. The plates were washed with 3x300ul/well TBST. Colour development was started by addition of 100 ul tetra-methyl benzidine/hydrogen peroxide reagent. The reaction was stopped after 5 minutes by addition of 100 ul/well 1M sulfuric acid and the optical density is measured by an optical reader (Microplate Reader 3550, BioRad) at 450 nm. For comparison mouse monoclonal antibodies were analysed in the same way, except using as revealing agent HRP-labelled anti-mouse Ig instead of anti-human Ig.



Employing specific of the above described heptapeptides derived from A $\beta$ , specific ELISA-tests as described herein above were carried out. Preferably, inventive antibodies comprise antibodies which show, as measured by optical densities, a signal to background ratio above "10" when their reactivity with an A-beta derived peptide (AEFRHD; amino acid 2 to 7 of A-beta) is compared to an non-related protein/peptide like BSA. Most preferably, the ratio of optical densities is above "5" for a corresponding reaction with at least one of the following three A $\beta$  derived peptides: (VFFAED; amino acid 18 to 23 of A $\beta$ ) or (FFAEDV; amino acid 19 to 24 of A $\beta$ ) or (LVFFAE; amino acid 17 to 22 of A $\beta$ ).

Corresponding results for the inventive parental and/or matured antibodies are shown in the following two tables:



MS-R #	Peptide 2-7 2-7/BSA	Peptide 17-22 17-22/BSA	Peptide 18-23 18-23/BSA	Peptide 19-24 19-24/BSA	Peptide-ratio 17-22/2-7	Peptide-ratio 18-23/2-7	Peptide-ratio 19-24/2-7
7	24	4	7	4	0.17	0.29	0.17
8	28	10	29	25	0.36	1.04	0.89
7.2	34	12	16	9	0.35	0.47	0.26
7.3	34	11	15	9	0.32	0.44	0.26
7.4	36	10	13	6	0.28	0.36	0.17
7.9	28	9	13	8	0.32	0.46	0.29
7.11	37	11	15	9	0.30	0.41	0.24
7.12	38	6	8	7	0.16	0.21	0.18
8.1	30	1	11	8	0.03	0.37	0.27
8.2	32	4	28	23	0.13	0.88	0.72
3.2H2	26	12	23	20	0.46	0.88	0.77
3.3H1	23	4	12	8	0.17	0.52	0.35
3.3H3	31	2	5	2	0.06	0.16	0.06
3.4H1	27	2	8	2	0.07	0.30	0.07
3.4H2	16	11	1	1	0.69	0.06	0.06
3.4H3	22	9	17	11	0.41	0.77	0.50
3.4H5	28	5	13	4	0.18	0.46	0.14
3.4H7	24	2	6	5	0.08	0.25	0.21
3.4H17	28	5	12	11	0.18	0.43	0.39
3.4L11	31	6	20	5	0.19	0.65	0.16
3.6H6	25	1	4	7	0.04	0.16	0.28
3.6H1	23	3	13	5	0.13	0.57	0.22

3.6H2	19	2	8	3	0.11	0.42	0.16
7.2H1	38	8	11	9	0.21	0.29	0.24
7.2H2	16	10	10	10	0.63	0.63	0.63
7.2H3	33	17	20	18	0.52	0.61	0.55
7.2H4	23	12	13	12	0.52	0.57	0.52
7.2H5	30	13	18	15	0.43	0.60	0.50
7.2L1	24	14	16	11	0.57	0.68	0.45
7.4H1	31	16	20	16	0.52	0.65	0.51
7.4H2	36	17	20	16	0.47	0.56	0.46
7.9H1	32	7	12	6	0.23	0.36	0.19
7.9H2	35	3	6	8	0.08	0.16	0.23
7.9H3	35	11	20	9	0.31	0.57	0.27
7.9H4	30	10	15	7	0.32	0.49	0.22
7.11H1	31	8	9	8	0.25	0.29	0.25
7.11H2	34	10	12	14	0.29	0.36	0.41
7.12L1	16	10	12	10	0.60	0.70	0.59
8.1H1	29	22	25	25	0.77	0.88	0.86
8.2H1	22	7	23	20	0.34	1.05	0.94
8.2L1	26	15	32	31	0.60	1.26	1.22

Table 5: Reactivity of MS-R Fabs with BSA-conjugated Abeta heptapeptides 2-7 (AEFRHD), 17-22 (LVFFAE), 18-23 (VFFAED) and 19-24 (FFAEDV). The ratios of the ELISA read-out (optical density) obtained with peptide-conjugated and non-conjugated BSA are given. The signal intensities obtained with the 17-22, 18-23 and 19-24 peptides in relation to the 2-7 peptide are also indicated.

MS-R IgG #	AEFRHD 2-7/BSA	LVFFAE 17-22/BSA	VFFAED 18-23/BSA	FFAEDV 19-24/BSA	Peptide-ratio 17-22/2-7	Peptide-ratio 18-23/2-7	Peptide-ratio 19-24/2-7
3.3	17	11	16	11	0.65	0.94	0.65
7.12	19	11	13	11	0.58	0.68	0.58
8.1	16	7	16	14	0.44	1.00	0.88
<i>Mouse mab</i>							
BAP-1	21	1	1	1	0.05	0.05	0.05
BAP-2	21	1	1	1	0.05	0.05	0.05
4G8	1	23	20	1	23	20	1
6E10	18	1	1	1	0.06	0.06	0.06
Amy 33	16	2	1	3	0.13	0.06	0.19

**Table 6:** Reactivity of MS-R IgGs and mouse monoclonal antibodies BAP-1, BAP-2, 4G8, 6E10 and Amy-33 with BSA-conjugated A $\beta$  heptapeptides 2-7 (AEFRHD), 17-22 (LVFFAE), 18-23 (VFFAED) and 19-24 (FFAEDV). The ratios of the ELISA read-out (optical density) obtained with peptide-conjugated and non-conjugated BSA are given. The signal intensities obtained with the 17-22, 18-23 and 19-24 peptides in relation to the 2-7 peptide are also indicated.

**Claims**

1. An antibody molecule capable of specifically recognizing two regions of the  $\beta$ -A4 peptide/A $\beta$ 4, wherein the first region comprises the amino acid sequence AEFRHDSGY as shown in SEQ ID NO: 1 or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLVFFAEDVG as shown in SEQ ID NO: 2 or a fragment thereof.
2. The antibody molecule of claim 1, wherein said antibody molecule recognizes at least two consecutive amino acids within the two regions of  $\beta$ -A4.
3. The antibody molecule of claim 1 or 2, wherein said antibody molecule recognizes in the first region an amino acid sequence comprising: AEFRHD, EF, EFR, FR, EFRHDSG, EFRHD or HDSG and in the second region an amino acid sequence comprising: HHQL, LV, LVFFAE, VFFAED or VFFA, FFAEDV.
4. The antibody molecule of any one of claims 1 to 3, wherein said antibody molecule comprises a variable  $V_H$ -region as encoded by a nucleic acid molecule as shown in a SEQ ID NO selected from the group consisting of SEQ ID NO: 3, 5 or 7 or a variable  $V_H$ -region as shown in the amino acid sequences depicted in SEQ ID NOs: 4, 6 or 8.
5. The antibody molecule of any one of claims 1 to 3, wherein said antibody molecule comprises a variable  $V_L$ -region as encoded by a nucleic acid molecule as shown in a SEQ ID NO selected from the group consisting of SEQ ID NO: 9, 11 or 13 or a variable  $V_L$ -region as shown in the amino acid sequences depicted in SEQ ID NOs: 10, 12 or 14.

6. The antibody molecule of any one of claims 1 to 5, wherein said antibody molecule comprises at least one CDR3 of an  $V_L$ -region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 15, 17 or 19 or at least one CDR3 amino acid sequence of an  $V_L$ -region as shown in SEQ ID NOs: 16, 18 or 20 and/or wherein said antibody molecule comprises at least one CDR3 of an  $V_H$ -region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 21, 23 or 25 or at least one CDR3 amino acid sequence of an  $V_H$ -region as shown in SEQ ID NOs: 22, 24 or 26.
7. The antibody molecule of any one of claims 1 to 6, wherein said antibody is selected from the group consisting of MSR-3, -7 and -8 or an affinity-maturized version of MSR-3, -7 or -8.
8. The antibody molecule of any one of claims 1 to 7, wherein said antibody molecule is a full antibody (immunoglobulin), a F(ab)-fragment, a F(ab)<sub>2</sub>-fragment, a single-chain antibody, a chimeric antibody, a CDR-grafted antibody, a bivalent antibody-construct, or a synthetic antibody.
9. The antibody molecule of any one of claims 1 to 8, wherein said at least two regions of  $\beta$ -A4 form a conformational epitope or a discontinuous epitope.
10. A nucleic acid molecule encoding an antibody molecule of any one of claims 1 to 9.
11. A vector comprising the nucleic acid molecule of claim 10.
12. A host cell comprising the vector of claim 11.
13. A method for the preparation of an antibody molecule of any one of claims 1 to 9 comprising culturing the host cell of claim 12 under conditions that allow synthesis of said antibody molecule and recovering said antibody molecule from said culture.

14. A composition comprising an antibody molecule of any one of claims 1 to 9 or an antibody molecule produced by the method of claim 13.
15. The composition of claim 14, which is a pharmaceutical or a diagnostic composition.
16. Use of an antibody molecule of any one of claims 1 to 9 or an antibody molecule produced by the method of claim 13, of a nucleic acid molecule of claim 10, of a vector of claim 11 or a host of claim 12 for the preparation of a pharmaceutical composition for the preparation and/or treatment of a disease associated with amyloidogenesis and/or amyloid-plaque formation.
17. Use of an antibody molecule of any one of claims 1 to 9 or an antibody molecule produced by the method of claim 13 for the preparation of a diagnostic composition for the detection of a disease associated with amyloidogenesis and/or amyloid-plaque formation.
18. Use of an antibody molecule of any one of claims 1 to 9 or an antibody molecule produced by the method of claim 13 for the preparation of a pharmaceutical composition for the disintegration of  $\beta$ -amyloid plaques.
19. Use of an antibody molecule of any one of claims 1 to 9 or an antibody molecule produced by the method of claim 13 for the preparation of a pharmaceutical composition for passive immunization against  $\beta$ -amyloid plaque formation.
20. The use of claims 16 or 17, wherein said disease is dementia, Alzheimer's disease, motor neuropathy, Down's syndrome, Creutzfeldt Jacob disease, hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's disease, ALS or neuronal disorders related to aging.
21. Kit comprising an antibody molecule of any one of claims 1 to 9, a nucleic acid molecule of claim 15, a vector of claim 16 or a host cell of claim 17.

22. A method for the optimization of an antibody molecule as defined in any one of claims 1 to 9 comprising the steps of
- (a) constructing a library of diversified Fab antibody fragments derived from an antibody comprising at least one CDR3 of an  $V_H$ -region as encoded by a nucleic acid molecule as shown in SEQ ID NOs: 21, 23 or 25 or at least one CDR3 amino acid sequence of an  $V_H$ -region as shown in SEQ ID NOs: 22, 24 or 26;
  - (b) testing the resulting Fab optimization library by panning against  $A\beta/A\beta_4$ ;
  - (c) identifying optimized clones; and
  - (d) expressing of selected, optimized clones.
23. The method of claim 22 further comprising a step (ca), whereby the optimized clones are further optimized by cassette mutagenesis
24. The method of claim 22 or 23, wherein said  $A\beta/A\beta_4$  in step (b) is aggregated  $A\beta/A\beta_4$ .
25. The method of any one of claims 22 to 24, wherein said identification in step (c) is carried out by koff-ranking.
26. A method for the preparation of a pharmaceutical composition comprising the steps of
- (a) optimization of an antibody according to the method of any one of claims 22 to 25; and
  - (b) formulating the optimized antibody/antibody molecule with an physiologically acceptable carrier.
27. A pharmaceutical composition prepared by the method of claim 26.

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### Abstract

The present invention relates to antibody molecules capable of specifically recognizing two regions of the  $\beta$ -A4 peptide, wherein the first region comprises the amino acid sequence AEFRHDSGY as shown in SEQ ID NO: 1 or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLVFFAEDVG as shown in SEQ ID NO: 2 or a fragment thereof. Furthermore, nucleic acid molecules encoding the inventive antibody molecules and vectors and hosts comprising said nucleic acid molecules are disclosed. In addition, the present invention provides for compositions, preferably pharmaceutical or diagnostic compositions, comprising the compounds of the invention as well as for specific uses of the antibody molecules, nucleic acid molecules, vectors or hosts of the invention.



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Fig. 1a

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Fig. 1a cont.

Framework 3										Framework 4									
CDR3										CDR3									
6	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
S	R	F	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E
D	R	F	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E
A	R	F	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E
D	R	F	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	P	E
D	R	F	S	G	S	K	S	G	T	S	A	S	L	A	I	T	G	L	Q
N	R	F	S	G	S	K	S	G	T	A	S	L	T	I	S	G	L	Q	A
E	R	F	S	G	S	N	S	G	N	T	A	T	L	T	I	S	G	T	Q

Framework 3										Framework 4									
CDR3										CDR3									
7	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
R	V	T	I	T	A	D	E	S	T	S	T	A	Y	M	E	L	S	S	L
R	V	T	M	T	R	D	T	S	I	S	T	A	Y	M	E	L	S	S	L
R	L	T	I	S	K	D	T	S	K	N	Q	V	V	L	T	M	T	N	M
R	F	T	I	S	R	D	N	S	K	N	T	L	Y	L	Q	M	N	S	L
R	V	T	I	S	V	D	T	S	K	N	Q	F	S	L	K	L	S	S	V
Q	V	T	I	S	A	D	K	S	I	S	T	A	Y	L	Q	W	S	S	L
R	I	T	I	N	P	D	T	S	K	N	Q	F	S	L	Q	L	N	S	V

## Sequence Summary of HuCAL-Fab1 Library

[illegible][illegible]

Fig. 1b cont.

Framework 2										Framework 3									
5										8									
4										7									
6										6									
3										1									
2										0									
1										9									
8										8									
7										7									
6										6									
5										5									
4										4									
3										3									
2										2									
1										1									
0										0									
8										8									
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**Fig. 1b cont.**

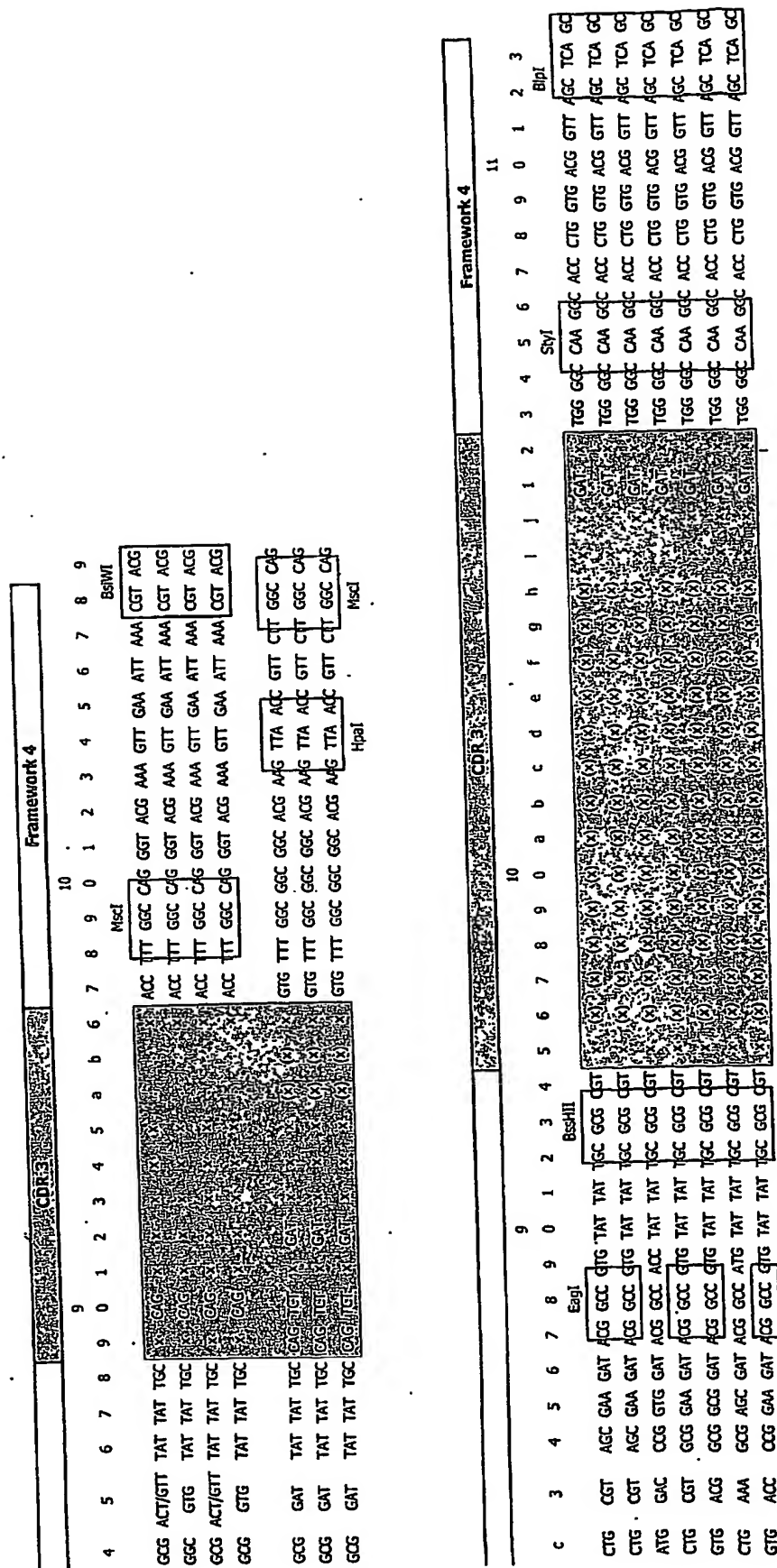
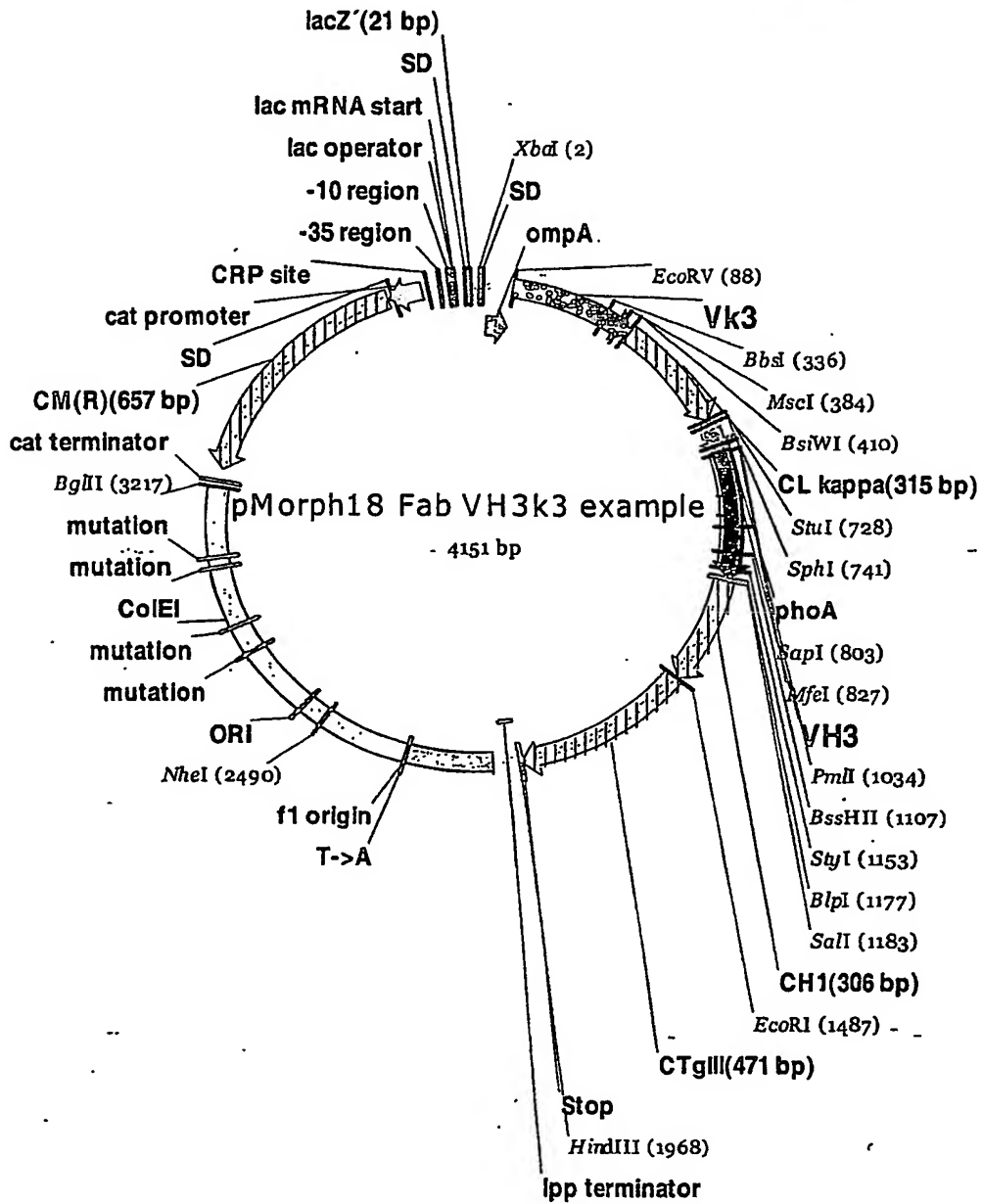


Fig. 2



```

lacZ'      SD      ompA
-----
XbaI
-----
1  TCTAGATAAC GAGGGCAAAA AATGAAAAAG ACAGCTATCG CGATTGCAGT
   AGATCTATTG CTCCCGTTTT TTACTTTTTC TGTCGATAGC GCTAACGTCA
                                   Vk3
                                   -----
                                   ompA
                                   -----
                                   EcoRV
                                   -----
51  A L A G F A T V A Q A D I V L T Q
    G G C A C T G G C T G G T T C G C T A C C G T A G C G C A G G C C G A T A T C G T G C T G A C C C

```

Fig. 2 cont.

CCGTGACCGA CCAAAGCGAT GGCATCGCGT CCGGCTATAG CACGACTGGG  
Vk3

101 S P A T L S L S P G E R A T L S  
AGAGCCCGGC GACCCTGAGC CTGTCTCCGG GCGAACGTGC GACCCTGAGC  
TCTCGGGCCG CTGGGACTCG GACAGAGGCC CGCTTGCACG CTGGGACTCG  
Vk3

151 C R A S Q S V S S S Y L A W Y Q Q  
TGCAGAGCGA GCCAGAGCGT GAGCAGCAGC TATCTGGCGT GGTACCAGCA  
ACGTCTCGCT CGGTCTCGCA CTCGTCTCGT ATAGACCGCA CCATGGTCGT  
Vk3

201 K P G Q A P R L L I Y G A S S R A  
GAAACCAGGT CAAGCACCGC GTCTATTAAT TTATGGCGCG AGCAGCCGTG  
CTTTGGTCCA GTTCGTGGCG CAGATAATTA AATACCGCGC TCGTCGGCAC  
Vk3

251 T G V P A R F S G S G S G T D F  
CAACTGGGGT CCCGGCGCGT TTTAGCGGCT CTGGATCCGG CACGGATTTT  
GTTGACCCCA GGGCCGCGCA AAATCGCCGA GACCTAGGCC GTGCCTAAAA  
Vk3

BbsI

301 T L T I S S L E P E D F A V Y Y C  
ACCCTGACCA TTAGCAGCCT GGAACCTGAA GACTTTGCGG TGTATTATTG  
TGGGACTGGT AATCGTCGGA CCTTGGACTT CTGAAACGCC ACATAATAAC  
Vk3

MscI

351 Q Q H Y T T P P T F G Q G T K V E  
CCAGCAGCAT TATACCACCC CGCCGACCTT TGGCCAGGGT ACGAAAGTTG  
GGTCGTCGTA ATATGGTGGG GCGGCTGGAA ACCGGTCCCA TGCTTCAAC  
CL kappa

Vk3

BsiWI

401 I K R T V A A P S V F I F P P S  
AAATTAAACG TACGGTGGCT GCTCCGAGCG TGTATTATTT TCCGCCGAGC  
TTTAATTTGC ATGCCACCGA CGAGGCTCGC ACAAATAAAA AGGCGGCTCG  
CL kappa

451 D E Q L K S G T A S V V C L L N N  
GATGAACAAC TGAAAAGCGG CACGGCGAGC GTGGTGTGCC TGCTGAACAA  
CTACTTGTG ACTTTTCGCC GTGCCGCTCG CACCACACGG ACGACTTGT  
CL kappa

501 F Y P R E A K V Q W K V D N A L Q  
CTTTTATCCG CGTGAAGCGA AAGTTCAGTG GAAAGTAGAC AACGCGCTGC  
GAAAATAGGC GCACTTCGCT TTCAAGTCAC CTTTCATCTG TTGCGCGACG  
CL kappa

551 S G N S Q E S V T E Q D S K D S  
AAAGCGGCAA CAGCCAGGAA AGCGTGACCG AACAGGATAG CAAAGATAGC  
TTTCGCCGTT GTCGGTCCTT TCGCACTGGC TTGTCCTATC GTTCTATCG  
CL kappa

## Fig. 2 cont.

```

      T Y S L S S T L T L S K A D Y E K
601  ACCTATTCTC TGAGCAGCAC CCTGACCCTG AGCAAAGCGG ATTATGAAAA
      TGGATAAGAG ACTCGTCTGT GGA CTGGGAC TCGTTTCGCC TAATACTTTT
              CL kappa
      ~~~~~
      H K V Y A C E V T H Q G L S S P V
651  ACATAAAGTG TATGCGTGCG AAGTGACCCA TCAAGGTCTG AGCAGCCCCG
      TGTATTTTAC ATACGCACGC TTCACTGGGT AGTTCAGAC TCGTCGGGCC
              CL kappa
      ~~~~~
                                StuI      SphI
                                ~~~~~
      T K S F N R G E A
701  TGACTAAATC TTTTAATCGT GGCAGGCCT GATAAGCATG CGTAGGAGAA
      ACTGATTTAG AAAATTAGCA CCGCTCCGGA CTATTCGTAC GCATCCTCTT
              phoA
      ~~~~~
                                SspI
                                ~~~~~
      M K Q S T I A L A L L P L L F
751  AATAAAATGA AACAAAGCAC TATTGCACTG GCACTCTTAC CGTTGCTCTT
      TTATTTTACT TTGTTTCGTG ATAACGTGAC CGTGAGAATG GCAACGAGAA
              VH3
      ~~~~~
      phoA
      ~~~~~
SspI                                MfeI
~
      T P V T K A Q V Q L V E S G G G L
801  CACCCCTGTT ACCAAAGCCG AAGTGCAATT GGTGGAAAGC GGC GGCGGCC
      GTGGGGACAA TGGTTTCGGC TTCACGTTAA CCACCTTTCG CCGCCGCCGG
              VH3
      ~~~~~
      V Q P G G S L R L S C A A S G F
851  TGGTGCAACC GGGCGGCAGC CTGCGTCTGA GCTGCGCGGC CTCCGGATTT
      ACCACGTTGG CCCGCCGTCG GACGCAGACT CGACGCGCCG GAGGCCATAA
              VH3
      ~~~~~
      T F S S Y A M S W V R Q A P G K G
901  ACCTTTAGCA GCTATGCGAT GAGCTGGGTG CGCCAAGCCC CTGGGAAGGG
      TGGAAATCGT CGATACGCTA CTCGACCCAC GCGGTTTCGGG GACCCTTCCC
              VH3
      ~~~~~
      L E W V S A I S G S G G S T Y Y A
951  TCTCGAGTGG GTGAGCGCGA TTAGCGGTAG CGGCGGCAGC ACCTATTATG
      AGAGCTCACC CACTCGCGCT AATCGCCATC GCCGCCGTCG TGGATAATAC
              VH3
      ~~~~~
                                PmlI
                                ~~~~~
      D S V K G R F T I S R D N S K N
1001 CGGATAGCGT GAAAGGCCGT TTTACCATTT CACGTGATAA TTCGAAAAAC
      GCCTATCGCA CTTTCCGGCA AAATGGTAAA GTGCACTATT AAGCTTTTGT
              VH3
      ~~~~~
      T L Y L Q M N S L R A E D T A V Y
1051 ACCCTGTATC TGCAAATGAA CAGCCTGCGT GCGGAAGATA CGGCCGTGTA
      TGGGACATAG ACGTTTACTT GTCGGACGCA CGCCTTCTAT GCCGGCACAT
              VH3
      ~~~~~
      BssHII

```



Fig. 2 cont.

1101 . Y C A R W G G D G F Y A M D Y W G .  
 TTATTGCGCG CGTTGGGGCG GCGATGGCTT TTATGCGATG GATTATTGGG  
 AATAACGCGC GCAACCCCGC CGCTACCGAA AATACGCTAC CTAATAACCC  
 CH1

VH3

Sall

StyI

BlnI

1151 . Q G T L V T V S S A S T K G P S  
 GCCAAGGCAC CCTGGTGACG GTTAGCTCAG CGTCGACCAA AGGTCCAAGC  
 CGGTTCCGTG GGACCACTGC CAATCGAGTC GCAGCTGGTT TCCAGGTTTC  
 CH1

1201 V F P L A P S S K S T S G G T A A  
 GTGTTTCCGC TGGCTCCGAG CAGCAAAAGC ACCAGCGGCG GCACGGCTGC  
 CACAAAGGCG ACCGAGGCTC GTCGTTTTCG TGGTCGCCGC CGTGCCGACG  
 CH1

1251 . L G C L V K D Y F P E P V T V S W .  
 CCTGGGCTGC CTGGTTAAAG ATTATTTCCC GGAACCAAGTC ACCGTGAGCT  
 GGACCCGACG GACCAATTTT TAATAAAGG CCTTGGTCAG TGGCACTCGA  
 CH1

1301 . N S G A L T S G V H T F P A V L  
 GGAACAGCGG GGCCTGACC AGCGGCGTGC ATACCTTTCC GGCAGTGTCTG  
 CCTTGTCGCC CCGCGACTGG TCGCCGACG TATGGAAAG CCGCCACGAC  
 CH1

1351 Q S S G L Y S L S S V V T V P S S  
 CAAAGCAGCG GCCTGTATAG CCTGAGCAGC GTTGTGACCG TGCCGAGCAG  
 GTTTCGTGCG CGGACATATC GGACTCGTCG CAACACTGGC ACGGCTCGTC  
 CH1

1401 . S L G T Q T Y I C N V N H K P S N  
 CAGCTTAGGC ACTCAGACCT ATATTTGCAA CGTGAACCAT AAACCGAGCA  
 GTCGAATCCG TGAGTCTGGA TATAAACGTT GCACTTGGTA TTTGGCTCGT  
 CH1 CTgIII

EcoRI

1451 . T K V D K K V E P K S E F G G G  
 ACACCAAAGT GGATAAAAAA GTGGAACCGA AAAGCGAATT CGGGGGAGGG  
 TGTGGTTTCA CCTATTTTTT CACCTTGGCT TTTCGCTTAA GCCCCCTCC  
 CTgIII

1501 S G S G D F D Y E K M A N A N K G  
 AGCGGGAGCG GTGATTTTGA TTATGAAAAG ATGGCAAACG CTAATAAGGG  
 TCGCCCTCGC CACTAAACT AATACTTTTC TACCGTTTGC GATTATTTCC  
 CTgIII

1551 . A M T E N A D E N A L Q S D A K G  
 GGCTATGACC GAAAATGCCG ATGAAAACGC GCTACAGTCT GACGCTAAAG  
 CCGATACTGG CTTTACGGC TACTTTTGC CGATGTCAGA CTGCGATTTC  
 CTgIII

1601 . K L D S V A T D Y G A A I D G F  
 GCAAACCTGA TTCTGTCGCT ACTGATTACG GTGCTGCTAT CGATGGTTTC

Fig. 2 cont.

```

CGTTTGAAGT AAGACAGCGA TGACTAATGC CACGACGATA GCTACCAAAG
CTgIII
-----
I G D V S G L A N G N G A T G D F
1651 ATTGGTGACG TTTCCGGCCT TGCTAATGGT AATGGTGCTA CTGGTGATTT
TAACCACTGC AAAGGCCGGA ACGATTACCA TTACCACGAT GACCACTAAA
CTgIII
-----
A G S N S Q M A Q V G D G D N S P
1701 TGCTGGCTCT AATTCCCAAA TGGCTCAAGT CGGTGACGGT GATAATTCAC
ACGACCGAGA TTAAGGGTTT ACCGAGTTCA GCCACTGCCA CTATTAAGTG
CTgIII
-----
L M N N F R Q Y L P S L P Q S V
1751 CTTTAATGAA TAATTTCCGT CAATATTTAC CTTCCCTCCC TCAATCGGTT
GAAATTACTT ATTAAAGGCA GTTATAAATG GAAGGGAGGG AGTTAGCCAA
CTgIII
-----
E C R P F V F G A G K P Y E F S I
1801 GAATGTCGCC CTTTGTCTT TGGCGCTGGT AAACCATATG AATTTTCTAT
CTTACAGCGG GAAAACAGAA ACCGCGACCA TTTGGTATAC TTAAAAGATA
CTgIII
-----
D C D K I N L F R G V F A F L L Y
1851 TGATTGTGAC AAAATAAACT TATTCCTGGT TGTCTTTGCG TTTCTTTTAT
ACTAACACTG TTTTATTTGA ATAAGGCACC ACAGAAACGC AAAGAAAATA
CTgIII
-----
V A T F M Y V F S T F A N I L R
1901 ATGTTGCCAC CTTTATGTAT GTATTTTCTA CGTTTGCTAA CATACTGCGT
TACAACGGTG GAAATACATA CATAAAAGAT GCAAACGATT GTATGACGCA
CTgIII
-----
Stop lpp terminator
-----
HindIII
-----
N K E S
1951 AATAAGGAGT CTTGATAAGC TTGACCTGTG AAGTGAAAAA TGGCGCAGAT
TTATTCTCA GAACTATTCT AACTGGACAC TTCCTTTTTT ACCGCGTCTA
lpp terminator
-----
2001 TGTGCGACAT TTTTTTTGTC TGCCGTTTAA TGAAATTGTA AACGTTAATA
ACACGCTGTA AAAAAACAG ACGGCAAATT ACTTTAACAT TTGCAATTAT
-----
f1 origin
2051 TTTTGTTAAA ATTCGCGTTA AATTTTGTG AAATCAGCTC ATTTTAAAC
AAAACAATTT TAAGCGCAAT TTAAAAACAA TTTAGTCGAG TAAAAAATTG
-----
f1 origin
2101 CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG AATAGACCGA
GTTATCCGGC TTTAGCCGTT TTAGGAATA TTTAGTTTTC TTATCTGGCT
-----
f1 origin
2151 GATAGGGTTG AGTGTGTGTC CAGTTTGGAA CAAGAGTCCA CTATTAAAGA
CTATCCCAAC TCACAACAAG GTCAAACCTT GTTCTCAGGT GATAATTTCT
-----
f1 origin
2201 ACGTGGACTC CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC
TGCACCTGAG GTTGCAGTTT CCCGCTTTTT GGCAGATAGT CCCGCTACCG
-----

```

## Fig. 2 cont.

f1 origin

T-&gt;A

2251 CCACTACGAG AACCATCACC CTAATCAAGT TTTTGGGGT CGAGGTGCCG  
GGTGATGCTC TTGGTAGTGG GATTAGTTCA AAAAACCCCA GCTCCACGGC

f1 origin

2301 TAAAGCACTA AATCGGAACC CTAAAGGGAG CCCCCGATTT AGAGCTTGAC  
ATTTTCGTGAT TTAGCCTTGG GATTTCCTC GGGGGCTAAA TCTCGAACTG

f1 origin

2351 GGGGAAAGCC GCGCAACGTG GCGAGAAAGG AAGGGAAGAA AGCGAAAGGA  
CCCCTTTCCG CCGCTTGCAC CGCTCTTCC TTCCCTTCTT TCGCTTTCTT

f1 origin

2401 GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG GTCACGCTGC GCGTAACCAC  
CGCCCGCGAT CCCGCGACCG TTCACATCGC CAGTGCACG CGCATTGGTG

f1 origin

NheI

2451 CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTGC TAGCCATGTG  
GTGTGGGCGG CGCGAATTAC GCGGCGATGT CCCGCGCACG ATCGGTACAC

f1 origin

CotEI

2501 AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG  
TCGTTTCCG GTCGTTTCC GGTCTTGGC ATTTTCCCG CGCAACGACC

CotEI

ORI

2551 CGTTTTTCCA TAGGCTCCGC CCCCTGACG AGCATCACAA AAATCGACGC  
GCAAAAAGGT ATCCGAGGCG GGGGGACTGC TCGTAGTGTT TTTAGCTGCG

CotEI

2601 TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAGAT ACCAGGCGTT  
AGTTCAGTCT CCACCGCTTT GGGCTGTCCT GATATTTCTA TGGTCCGCAA

CotEI

2651 TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA  
AGGGGGACCT TCGAGGGAGC ACGCGAGAGG ACAAGGCTGG GACGGCGAAT

CotEI

mutation

2701 CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTCTCAT  
GGCCTATGGA CAGGCGGAAA GAGGGAAGCC CTTCGCACCG CGAAAGAGTA

CotEI

mutation

2751 AGCTCACGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTT GCTCCAAGCT  
TCGAGTGCGA CATCCATAGA GTCAAGCCAC ATCCAGCAAG CGAGGTTCTA

CotEI

mutation

2801 GGGCTGTGTG CACGAACCCC CCGTTCAGTC CGACCGCTGC GCCTTATCCG  
CCCGACACAC GTGCTTGGGG GGCAAGTCAG GCTGGCGACG CGGAATAGGC

CotEI

Fig. 2 cont.

```

2851  GTAACATCG  TCTTGAGTCC  AACCCGGTAA  GACACGACTT  ATGCCCCTG
      CATTGATAGC  AGAACTCAGG  TTGGGCCATT  CTGTGCTGAA  TAGCGGTGAC
      ~~~~~~
                        ColeI
2901  GCAGCAGCCA  CTGGTAACAG  GATTAGCAGA  GCGAGGTATG  TAGGCGGTGC
      CGTCGTCGGT  GACCATTGTC  CTAATCGTCT  CGCTCCATAC  ATCCGCCACG
      ~~~~~~
                        ColeI
                                                mutation
2951  TACAGAGTTC  TTGAAGTGGT  GGCCTAACTA  CGGCTACACT  AGAAGAACAG
      ATGTCTCAAG  AACTTCACCA  CCGGATTGAT  GCCGATGTGA  TCTTCTTGTC
      ~~~~~~
                        ColeI
                        mutation
3001  TATTTGGTAT  CTGCGCTCTG  CTGTAGCCAG  TTACCTTCGG  AAAAAGAGTT
      ATAAACCATA  GACGCGAGAC  GACATCGGTC  AATGGAAGCC  TTTTCTCAA
      ~~~~~~
                        ColeI
3051  GGTAGCTCTT  GATCCGGCAA  ACAAACCACC  GCTGGTAGCG  GTGGTTTTTT
      CCATCGAGAA  CTAGGCCGTT  TGTTTGGTGG  CGACCATCGC  CACCAAAAAA
      ~~~~~~
                        ColeI
3101  TGTTTGCAAG  CAGCAGATTA  CGCGCAGAAA  AAAAGGATCT  CAAGAAGATC
      ACAAACGTTT  GTCGTCTAAT  GCGCGTCTTT  TTTTCTTAGA  GTTCTTCTAG
      ~~~~~~
                        ColeI
3151  CTTTGATCTT  TTCTACGGGG  TCTGACGCTC  AGTGAACGA  AAATCAGCT
      GAACTAGAA  AAGATGCCCC  AGACTGCGAG  TCACCTTGCT  TTTGAGTGCA
      ~~~~~~
                        ColeI
                                                cat terminator
                        ~~~~~~
                        BglII
                        ~~~~~~
3201  TAAGGGATTT  TGGTCAGATC  TAGCACCAGG  CGTTTAAGGG  CACCAATAAC
      ATTCCTTAAA  ACCAGTCTAG  ATCGTGGTCC  GCAAATTCCC  GTGGTTATTG
      ~~~~~~
                        ColeI
                        cat terminator
                        ~~~~~~
3251  TGCCTTAAAA  AAATTACGCC  CCGCCCTGCC  ACTCATCGCA  GTACTGTTGT
      ACGGAATTTT  TTTAATGCGG  GCGGGACGG  TGAGTAGCGT  CATGACAACA
      ~~~~~~
                        CM(R)
3301  AATTCATTAA  GCATTCTGCC  GACATGGAAG  CCATCACAAA  CGGCATGATG
      TTAAGTAATT  CGTAAGACGG  CTGTACCTTC  GGTAGTGTTT  GCCGTACTAC
      ~~~~~~
                        CM(R)
3351  AACCTGAATC  GCCAGCGGCA  TCAGCACCTT  GTCGCCTTGC  GTATAATATT
      TTGGACTTAG  CGGTCGCCGT  AGTCGTGGAA  CAGCGGAACG  CATATTATAA
      ~~~~~~
                        CM(R)
3401  TGCCCATAGT  GAAAACGGGG  GCGAAGAAGT  TGTCCATATT  GGCTACGTTT
      ACGGGTATCA  CTTTGGCCCC  CGTTCTTCA  ACAGGTATAA  CCGATGCAAA
      ~~~~~~
                        CM(R)
3451  AAATCAAAAC  TGGTGAAACT  CACCCAGGGA  TTGGCTGAGA  CGAAAAACAT
      TTTAGTTTTG  ACCACTTTGA  GTGGGTCCCT  AACCGACTCT  GCTTTTTGTA
      ~~~~~~

```

Fig. 2 cont.

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                                CM(R)
3501  ATTCTCAATA AACCCTTTAG GGAAATAGGC CAGGTTTTCA CCGTAACACG
      TAAGAGTTAT TTGGGAAATC CCTTTATCCG GTCCAAAAGT GGCATTGTGC
      ~~~~~

                                CM(R)
3551  CCACATCTTG CGAATATATG TGTAGAAACT GCCGGAAATC GTCGTGGTAT
      GGTGTAGAAC GCTTATATAC ACATCTTTGA CGGCCTTTAG CAGCACCATA
      ~~~~~

                                CM(R)
3601  TCACTCCAGA GCGATGAAAA CGTTTCAGTT TGCTCATGGA AAACGGTGTA
      AGTGAGGTCT CGCTACTTTT GCAAAGTCAA ACGAGTACCT TTTGCCACAT
      ~~~~~

                                CM(R)
3651  ACAAGGGTGA ACACTATCCC ATATCACCAG CTCACCGTCT TTCATTGCCA
      TGTTCCTACT TGTGATAGGG TATAGTGGTC GAGTGGCAGA AAGTAACGGT
      ~~~~~

                                CM(R)
3701  TACGGAACTC CGGGTGAGCA TTCATCAGGC GGGCAAGAAT GTGAATAAAG
      ATGCCTTGAG GCCCACTCGT AAGTAGTCCG CCCGTTCTTA CACTTATTTT
      ~~~~~

                                CM(R)
3751  GCCGGATAAA ACTTGTGCTT ATTTTCTTTT ACGGTCTTTA AAAAGGCCCGT
      CGGCCTATTT TGAACACGAA TAAAAAGAAA TGCCAGAAAT TTTCCGGCA
      ~~~~~

                                CM(R)
3801  AATATCCAGC TGAACGGTCT GGTTATAGGT ACATTGAGCA ACTGACTGAA
      TTATAGGTCG ACTTGCCAGA CCAATATCCA TGAACTCGT TGA CTGACTT
      ~~~~~

                                CM(R)
3851  ATGCCTCAAA ATGTTCTTTA CGATGCCATT GGGATATATC AACGGTGGTA
      TACGGAGTTT TACAAGAAAT GCTACGGTAA CCCTATATAG TTGCCACCAT
      ~~~~~

                                CM(R)
3901  TATCCAGTGA TTTTTTCTC CATTTTAGCT TCCTTAGCTC CTGAAAATCT
      ATAGGTCACT AAAAAAGAG GTAAAATCGA AGGAATCGAG GACTTTTAGA
      ~~~~~

                                CM(R)                                SD
                                ~~~~~

                                cat promoter
3951  CGATAACTCA AAAAATACGC CCGGTAGTGA TCTATTTTCA TTATGGTGAA
      GCTATTGAGT TTTTATGCG GGCCATCACT AGAATAAAGT AATACCACTT
      ~~~~~

                                cat promoter
                                CRP site
                                ~~~~~
4001  AGTTGGAACC TCACCCGACG TCTAATGTGA GTTAGCTCAC TCATTAGGCA
      TCAACCTTGG AGTGGGCTGC AGATTACACT CAATCGAGTG AGTAATCCGT
      ~~~~~
      cat promoter
                                lac mRNA
      start \
                                lac operator
                                ~~~~~

                                -35 region                                -10 region
                                ~~~~~                                ~~~~~
4051  CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT GTGGAATTGT
      GGGGTCCGAA ATGTGAAATA CGAAGGCCGA GCATACAACA CACCTTAACA
      lac operator                                SD                                lacZ'
      ~~~~~                                ~~~~~

4101  GAGCGGATAA CAATTCACA CAGGAAACAG CTATGACCAT GATTACGAAT

```

## Fig. 2 cont.

CTCGCCTATT GTTAAAGTGT GTCCTTTGTC GATACTGGTA CTAATGCTTA  
lacZ'

4151

-  
T  
A

Fig. 3

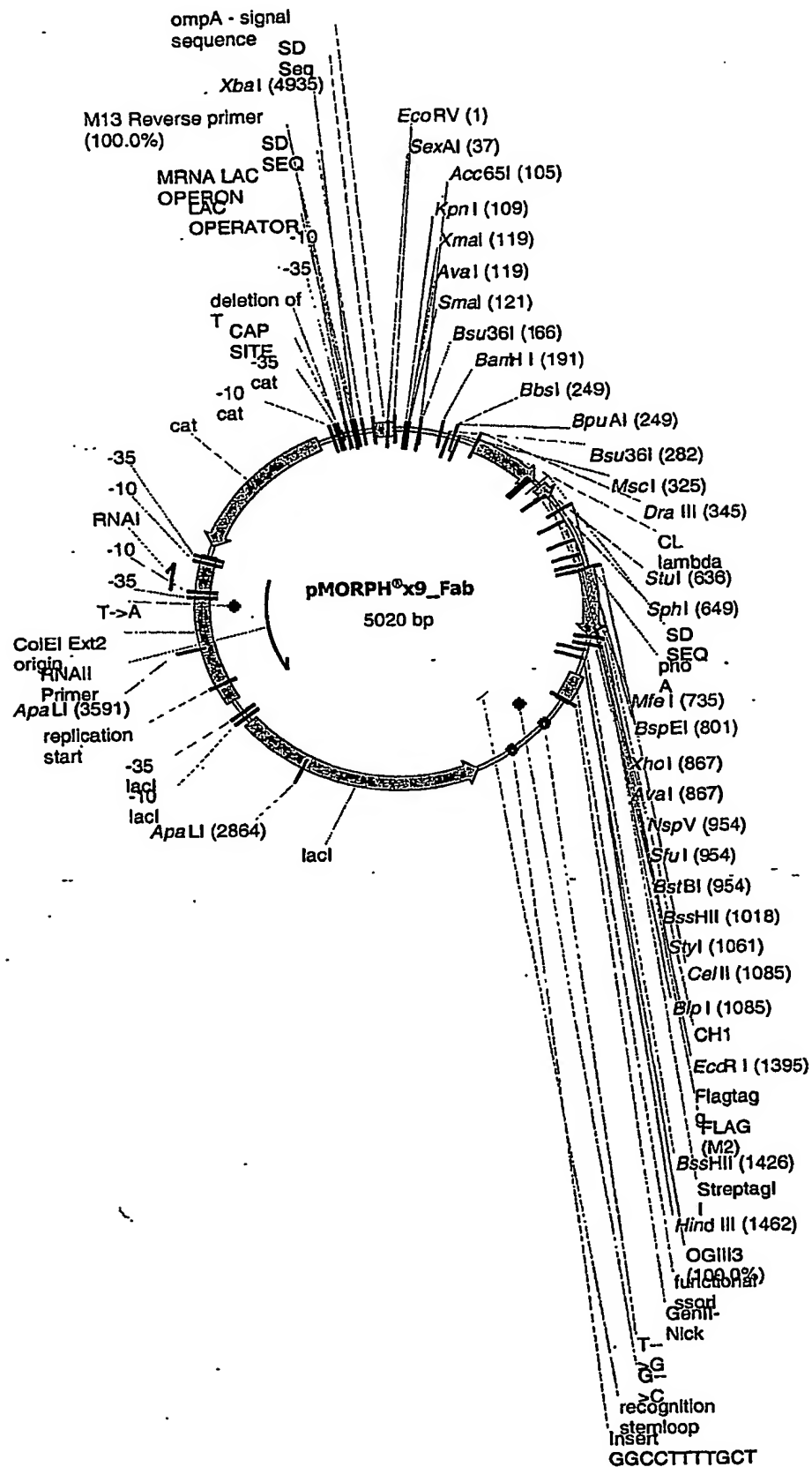


Fig. 3 cont.

EcoRV		SexAI	
~~~~~		~~~~~	
1	ATCGTGCTGA CCCAGCCGCC TTCAGTGAGT GGCACACCAG GTCAGCGTGT TAGCACGACT GGGTCGGCGG AAGTCACTCA CCGCGTGGTC CAGTCGCACA		
51	GACCATCTCG TGTAGCGGCA GCAGCAGCAA CATTTGGCAGC AACTATGTGA CTGGTAGAGC ACATCGCCGT CGTCGTCGTT GTAACCGTCG TTGATACACT		
		XmaI	
		~~~~~	
	KpnI	SmaI	
	~~~~~	~~~~~	
	Acc65I	AvaI	
	~~~~~	~~~~~	
101	GCTGGTACCA GCAGTTGCCC GGGACGGCGC CGAAACTGCT GATTTATGAT CGACCATGGT CGTCAACGGG CCCTGCCGCG GCTTTGACGA CTAAATACTA		
		Bsu36I	BamHI
		~~~~~	~~~~~
151	AACAACCAGC GTCCCTCAGG CGTGCCGGAT CGTTTTAGCG GATCCAAAAG TTGTTGGTCG CAGGGAGTCC GCACGGCCTA GCAAATCGC CTAGGTTTTTC		
			BpuAI
			~~~~~
			BbsI
			~~~~~
201	CGGCACCAGC GCGAGCCTTG CGATTACGGG CCTGCAAAGC GAAGACGAAG GCCGTGGTCG CGCTCGGAAC GCTAATGCCC GGACGTTTCG CTTCTGCTTC		
		Bsu36I	
		~~~~~	
251	CGGATTATTA TTGCCAGAGC TATGACATGC CTCAGGCTGT GTTTGGCGGC GCCTAATAAT AACGGTCTCG ATACTGTACG GAGTCCGACA CAAACCGCCG		
		MscI	DraIII
		~~~~~	~~~~~
301	GGCACGAAGT TTAACCGTTC TTGGCCAGCC GAAAGCCGCA CCGAGTGTGA CCGTGCTTCA AATTGGCAAG AACCGGTCGG CTTTCGGCGT GGCTCACACT		
351	CGCTGTTTCC GCCGAGCAGC GAAGAATTGC AGGCGAACAA AGCGACCCTG GCGACAAAGG CGGCTCGTCG CTTCTTAACG TCCGCTTGTT TCGCTGGGAC		
401	GTGTGCCTGA TTAGCGACTT TTATCCGGGA GCCGTGACAG TGGCCTGGAA CACACGGACT AATCGCTGAA AATAGGCCCT CGGCACTGTC ACCGGACCTT		
451	GGCAGATAGC AGCCCCGTCA AGGCGGGAGT GGAGACCACC ACACCCTCCA CCGTCTATCG TCGGGGCAGT TCCGCCCTCA CCTCTGGTGG TGTGGGAGGT		
501	AACAAAGCAA CAACAAGTAC GCGGCCAGCA GCTATCTGAG CCTGACGCCT TTGTTTCGTT GTTGTTTCATG CGCCGGTCGT CGATAGACTC GGACTGCGGA		
551	GAGCAGTGGA AGTCCCACAG AAGCTACAGC TGCCAGGTCA CGCATGAGGG CTCGTCACCT TCAGGGTGTC TTCGATGTCTG ACGGTCCAGT GCGTACTCCC		



				StuI	SphI
				~~~~~	~~~~~
601	GAGCACCGTG	GAAAAAACCG	TTGCGCCGAC	TGAGGCCTGA	TAAGCATGCG
	CTCGTGGCAC	CTTTTTTGGC	AACGCGGCTG	ACTCCGGACT	ATTCGTACGC
651	TAGGAGAAAA	TAAAATGAAA	CAAAGCACTA	TTGCACTGGC	ACTCTTACCG
	ATCCTCTTTT	ATTTTACTTT	GTTTCGTGAT	AACGTGACCG	TGAGAATGGC
			MfeI	~~~~~	
701	TTGCTCTTCA	CCCCTGTTAC	CAAAGCCCAG	GTGCAATTGA	AAGAAAGCGG
	AACGAGAAGT	GGGACAATG	GTTTCGGGTC	CACGTTAAC	TTCTTTCGCC
					BspEI
751	CCCGGCCCTG	GTGAAACCGA	CCCAAACCCT	GACCCTGACC	TGTACCTTTT
	GGGCCGGGAC	CACCTTGGCT	GGGTTTGGGA	CTGGGACTGG	ACATGGAAAA
					BspEI
801	CCGGATTTAG	CCTGTCCACG	TCTGGCGTTG	GCGTGGGCTG	GATTCGCCAG
	GGCCTAAATC	GGACAGGTGC	AGACCGCAAC	CGCACCCGAC	CTAAGCGGTC
			XhoI	~~~~~	
			AvaI	~~~~~	
851	CCGCCTGGGA	AAGCCCTCGA	GTGGCTGGCT	CTGATTGATT	GGGATGATGA
	GGCGGACCCT	TTCGGGAGCT	CACCGACCGA	GACTAACTAA	CCCTACTACT
901	TAAGTATTAT	AGCACCAGCC	TGAAAACGCG	TCTGACCATT	AGCAAAGATA
	ATTCATAATA	TCGTGGTCGG	ACTTTTGCGC	AGACTGGTAA	TCGTTTCTAT
			BstBI	~~~~~	
			SfuI	~~~~~	
			NspV	~~~~~	
951	CTTCGAAAAA	TCAGGTGGTG	CTGACTATGA	CCAACATGGA	CCCGGTGGAT
	GAAGCTTTTT	AGTCCACCAC	GACTGATACT	GGTTGTACCT	GGGCCACCTA
			BssHII	~~~~~	
1001	ACGGCCACCT	ATTATTGCGC	GCGTTCTCCT	CGTTATCGTG	GTGCTTTTGA
	TGCCGGTGGA	TAATAACGCG	CGCAAGAGGA	GCAATAGCAC	CACGAAAACT
			StyI	~~~~~	
			BlpI	~~~~~	
			CelII	~~~~~	
1051	TTATTGGGGC	CAAGGCACCC	TGGTGACGGT	TAGCTCAGCG	TCGACCAAAG
	AATAACCCCG	GTTCCGTGGG	ACCACTGCCA	ATCGAGTCGC	AGCTGGTTTC

1101 GTCCAAGCGT GTTTCCGCTG GCTCCGAGCA GCAAAAGCAC CAGCGGCGGC  
 CAGGTTTCGCA CAAAGGCGAC CGAGGCTCGT CGTTTTTCGTG GTCGCCGCCG

1151 ACGGCTGCCC TGGGCTGCCT GGTTAAAGAT TATTTCCCGG AACCAGTCAC  
 TGCCGACGGG ACCCGACGGA CCAATTTCTA ATAAAGGGCC TTGGTCAGTG

1201 CGTGAGCTGG AACAGCGGGG CGCTGACCAG CGGCGTG CAT ACCTTTCCGG  
 GCACTCGACC TTGTCGCCCC GCGACTGGTC GCCGCACGTA TGGAAAGGCC

1251 CGGTGCTGCA AAGCAGCGGC CTGTATAGCC TGAGCAGCGT TGTGACCGTG  
 GCCACGACGT TTCGTCGCCG GACATATCGG ACTCGTCGCA AACTGGCAC

1301 CCGAGCAGCA GCTTAGGCAC TCAGACCTAT ATTTGCAACG TGAACCATAA  
 GGCTCGTCGT CGAATCCGTG AGTCTGGATA TAAACGTTGC ACTTGGTATT

EcoRI  
~~~~~

1351 ACCGAGCAAC ACCAAAGTGG ATAAAAAAGT GGAACCGAAA AGCGAATTCG  
 TGGCTCGTTG TGGTTTCACC TATTTTTTCA CCTTGGCTTT TCGCTTAAGC

BssHII  
~~~~~

1401 ACTATAAAGA TGACGATGAC AAAGGCGCGC CGTGGAGCCA CCCGCAGTTT  
 TGATATTTCT ACTGCTACTG TTTCCGCGCG GCACCTCGGT GGGCGTCAAA

HindIII  
~~~~~

1451 GAAAAATGAT AAGCTTGACC TGTGAAGTGA AAAATGGCGC AGATTGTGCG  
 CTTTTTACTA TTCGAAGTGG ACACTTCACT TTTTACCGCG TCTAACACGC

OGIII3 100.0%  
=====

1501 ACATTTTTTTT TGTCTGCCGT TTAATTAAAG GGGGGGGGGG GCCGGCCTGG  
 TGTAAAAAAA ACAGACGGCA AATTAATTTT CCCCCCCCCC CGGCCGGACC

1551 GGGGGGGTGT ACATGAAATT GTAAACGTTA ATATTTTGTT AAAATTCGCG  
 CCCCCCACA TGTACTTTAA CATTTGCAAT TATAAAACAA TTTTAAGCGC

1601 TTAAATTTTTT GTTAAATCAG CTCATTTTTTT AACCAATAGG CCGAAATCGG  
 AATTTAAAAA CAATTTAGTC GAGTAAAAAA TTGGTTATCC GGCTTTAGCC

1651 CAAAATCCCT TATAAATCAA AAGAATAGAC CGAGATAGGG TTGAGTGTG  
 GTTTTAGGGA ATATTTAGTT TTCTTATCTG GCTCTATCCC AACTCACAAC

1701 TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAACGTGGA CTCCAACGTC  
 AAGGTCAAAC CTTGTTCTCA GGTGATAATT TCTTGCACCT GAGGTTGCAG

1751 AAAGGGCGAA AAACCGTCTA TCAGGGCGAT GGCCCACTAC GAGAACCATC  
 TTTCCCGCTT TTTGGCAGAT AGTCCCGCTA CCGGGTGATG CTCTTGGTAG

1801 ACCCTAATCA AGTTTTTTGG GGTCGAGGTG CCGTAAAGCA CTAAATCGGA  
 TGGGATTAGT TCAAAAAACC CCAGCTCCAC GGCATTTTCGT GATTTAGCCT

1851 ACCCTAAAGG GAGCCCCCGA TTTAGAGCTT GACGGGGAAA GCCGGCGAAC  
 TGGGATTTCC CTCGGGGGCT AAATCTCGAA CTGCCCCTTT CGGCCGCTTG  
 1901 GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG CTAGGGCGCT  
 CACCGCTCTT TCCTTCCCTT CTTTCGCTTT CCTCGCCCGC GATCCCGCGA  
 1951 GGCAAGTGTA GCGGTCACGC TGCGCGTAAC CACCACACCC GCCGCGCTTA  
 CCGTTCACAT CGCCAGTGCG ACGCGCATTG GTGGTGTGGG CGGCGCGAAT  
 2001 ATGCGCCGCT ACAGGGCGCG TGCTAGACTA GTGTTTAAAC CGGACCGGGG  
 TACGCGGCGA TGTCCCGCGC ACGATCTGAT CACAAATTTG GCCTGGCCCC  
 2051 GGGGGCTTAA GTGGGCTGCA AAACAAAACG GCCTCCTGTC AGGAAGCCGC  
 CCCCCGAATT CACCCGACGT TTTGTTTTGC CGGAGGACAG TCCTTCGGCG  
 2101 TTTTATCGGG TAGCCTCACT GCCCGCTTTC CAGTCGGGAA ACCTGTCGTG  
 AAAATAGCCC ATCGGAGTGA CGGGCGAAAG GTCAGCCCTT TGGACAGCAC  
 2151 CCAGCTGCAT CAGTGAATCG GCCAACGCGC GGGGAGAGGC GGTTCGCGTA  
 GGTGACGTA GTCACCTAGC CGGTTGCGCG CCCCTCTCCG CCAAACGCAT  
 2201 TTGGGAGCCA GGGTGGTTTT TCTTTTCACC AGTGAGACGG GCAACAGCTG  
 AACCCTCGGT CCCACCAAAA AGAAAAGTGG TCACTCTGCC CGTTGTGCGAC  
 2251 ATTGCCCTTC ACCGCCTGGC CCTGAGAGAG TTGCAGCAAG CGGTCCACGC  
 TAACGGGAAG TGGCGGACCG GGACTCTCTC AACGTCGTTT GCCAGGTGCG  
 2301 TGGTTTGCCC CAGCAGGCGA AAATCCTGTT TGATGGTGGT CAGCGGCGGG  
 ACCAAACGGG GTCGTCCGCT TTTAGGACAA ACTACCACCA GTCGCCGCCC  
 2351 ATATAACATG AGCTGTCCTC GGTATCGTCG TATCCCACTA CCGAGATGTC  
 TATATTGTAC TCGACAGGAG CCATAGCAGC ATAGGGTGAT GGCTCTACAG  
 2401 CGCACCAACG CGCAGCCCGG ACTCGGTAAT GGCACGCATT GCGCCCAGCG  
 GCGTGGTTGC GCGTCGGGCC TGAGCCATTA CCGTGCGTAA CGCGGGTCGC  
 2451 CCATCTGATC GTTGGCAACC AGCATCGCAG TGGGAACGAT GCCCTCATTC  
 GGTAGACTAG CAACCGTTGG TCGTAGCGTC ACCCTTGCTA CGGGAGTAAG  
 2501 AGCATTTGCA TGGTTTGTTG AAAACCGGAC ATGGCACTCC AGTCGCCTTC  
 TCGTAAACGT ACCAAACAAC TTTTGGCCTG TACCGTGAGG TCAGCGGAAG  
 2551 CCGTTCCGCT ATCGGCTGAA TTTGATTGCG AGTGAGATAT TTATGCCAGC  
 GGCAAGGCGA TAGCCGACTT AAATAACGC TCACTCTATA AATACGGTCG  
 2601 CAGCCAGACG CAGACGCGCC GAGACAGAAC TTAATGGGCC AGCTAACAGC  
 GTCGGTCTGC GTCTGCGCGG CTCTGTCTTG AATTACCCGG TCGATTGTCTG  
 2651 GCGATTTGCT GGTGGCCCAA TGCAGCCAGA TGCTCCACGC CCAGTCGCGT  
 CGCTAAACGA CCACCGGGTT ACGCTGGTCT ACGAGGTGCG GGTCAGCGCA  
 2701 ACCGTCCTCA TGGGAGAAAA TAATACTGTT GATGGGTGTC TGGTCAGAGA  
 TGGCAGGAGT ACCCTCTTTT ATTATGACAA CTACCCACAG ACCAGTCTCT

2751 CATCAAGAAA TAACGCCGGA ACATTAGTGC AGGCAGCTTC CACAGCAATA  
GTAGTTCTTT ATTGCGGCCT TGTAATCACG TCCGTCGAAG GTGTCGTTAT

2801 GCATCCTGGT CATCCAGCGG ATAGTTAATA ATCAGCCCAC TGACACGTTG  
CGTAGGACCA GTAGGTCGCC TATCAATTAT TAGTCGGGTG ACTGTGCAAC

ApaLI  
~~~~~

2851 CGCGAGAAGA TTGTGCACCG CCGCTTTACA GGCTTCGACG CCGCTTCGTT  
GCGCTCTTCT AACACGTGGC GGCAGAAATGT CCGAAGCTGC GGCAGCAAC

2901 CTACCATCGA CACGACCACG CTGGCACCCA GTTGATCGGC GCGAGATTTA  
GATGGTAGCT GTGCTGGTGC GACCGTGGGT CAACTAGCCG CGCTCTAAAT

2951 ATCGCCGCGA CAATTTGCGA CGGCGCGTGC AGGGCCAGAC TGGAGGTGGC  
TAGCGGCGCT GTTAAACGCT GCCGCGCACG TCCCGGTCTG ACCTCCACCG

3001 AACGCCAATC AGCAACGACT GTTTGCCCGC CAGTTGTTGT GCCACGCGGT  
TTGCGGTTAG TCGTTGCTGA CAAACGGGCG GTCAACAACA CGGTGCGCCA

3051 TAGGAATGTA ATTCAGCTCC GCCATCGCCG CTTCCACTTT TTCCCGCGTT  
ATCCTTACAT TAAGTCGAGG CGGTAGCGGC GAAGGTGAAA AAGGGCGCAA

3101 TTCGCAGAAA CGTGGCTGGC CTGGTTCACC ACGCGGGAAA CGGTCTGATA  
AAGCGTCTTT GCACCGACCG GACCAAGTGG TCGGCCCTTT GCCAGACTAT

3151 AGAGACACCG GCATACTCTG CGACATCGTA TAACGTTACT GGTTCACAT  
TCTCTGTGGC CGTATGAGAC GCTGTAGCAT ATTGCAATGA CCAAAGTGTA

3201 TCACCACCCT GAATTGACTC TCTTCCGGGC GCTATCATGC CATAACGCGA  
AGTGGTGGGA CTTAACTGAG AGAAGGCCCG CGATAGTACG GTATGGCGCT

3251 AAGGTTTTGC GCCATTTCGAT GCTAGCCATG TGAGCAAAAG GCCAGCAAAA  
TTCCAAAACG CGGTAAGCTA CGATCGGTAC ACTCGTTTTTC CGGTCGTTTT

3301 GGCCAGGAAC CGTAAAAGG CCGCGTTGCT GGCCTTTTTTC CATAGGCTCC  
CCGGTCCTTG GCATTTTTTC GGCAGAACGA CCGCAAAAAG GTATCCGAGG

3351 GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA  
CGGGGGGACT GCTCGTAGTG TTTTATAGCTG CGAGTTCAGT CTCCACCGCT

3401 AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT  
TTGGGCTGTC CTGATATTTT TATGGTCCGC AAAGGGGGAC CTTGAGGGGA

3451 CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT  
GCACGCGAGA GGACAAGGCT GGGACGGCGA ATGGCCTATG GACAGGCGGA

3501 TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT  
AAGAGGGAAG CCCTTCGCAC CGCGAAAGAG TATCGAGTGC GACATCCATA

ApaLI  
~~~~~

3551 CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC  
GAGTCAAGCC ACATCCAGCA AGCGAGGTTC GACCCGACAC ACGTGCTTGG

## Fig. 3 cont.

3601 CCCC GTTCAG CCCG ACCGCT GCGC CTTATC CGGTAACTAT CGTCTTGAGT  
 GGGGCAAGTC GGGCTGGCGA CGCGGAATAG GCCATTGATA GCAGAACTCA  
 3651 CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC  
 GGTTGGGCCA TTCTGTGCTG AATAGCGGTG ACCGTCGTCG GTGACCATTG  
 3701 AGGATTAGCA GAGCGAGGTA TG TAGGCGGT GCTACAGAGT TCTTGAAGTG  
 TCCTAATCGT CTCGCTCCAT ACATCCGCCA CGATGTCTCA AGAACTTCAC  
 3751 GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC  
 CACCGGATTG ATGCCGATGT GATCTTCTTG TCATAAACCA TAGACGCGAG  
 3801 TGCTGTAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC  
 ACGACATCGG TCAATGGAAG CCTTTTCTC AACCATCGAG AACTAGGCCG  
 3851 AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT  
 TTTGTTTGGT GGCGACCATC GCCACCAAAA AAACAAACGT TCGTCGTCTA  
 3901 TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG  
 ATGCGCGTCT TTTTTCCTA GAGTTCTTCT AGGAAACTAG AAAAGATGCC  
 3951 GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCAGA  
 CCAGACTGCG AGTCACCTTG CTTTGTAGTG CAATTCCTA AAACCAGTCT  
 4001 TCTAGCACCA GGCGTTTAAG GGCACCAATA ACTGCCTTAA AAAAATTACG  
 AGATCGTGGT CCGCAAATC CCGTGGTTAT TGACGGAATT TTTTAAATGC  
 4051 CCCC GCCCTG CCACTCATCG CAGTACTGTT GTAATTCATT AAGCATTCTG  
 GGGGCGGGAC GGTGAGTAGC GTCATGACAA CATTAAAGTAA TTCGTAAGAC  
 4101 CCGACATGGA AGCCATCACA AACGGCATGA TGAACCTGA TCGCCAGCGG  
 GGCTGTACCT TCGGTAGTGT TTGCCGTACT ACTTGGA CT AGCGGTCGCC  
 4151 CATCAGCACC TTGTCGCCTT GCGTATAATA TTTGCCCATA GTGAAAACGG  
 GTAGTCGTGG AACAGCGGAA CGCATATTAT AAACGGGTAT CACTTTTGCC  
 4201 GGGCGAAGAA GTTGTCATA TTGGCTACGT TTAAATCAAA ACTGGTGAAA  
 CCCGCTTCTT CAACAGGTAT AACCGATGCA AATTTAGTTT TGACCACTTT  
 4251 CTCACCCAGG GATTGGCTGA GACGAAAAAC ATATTCTCAA TAAACCCTTT  
 GAGTGGGTCC CTAACCGACT CTGCTTTTTG TATAAGAGTT ATTTGGGAAA  
 4301 AGGGAAATAG GCCAGGTTTT CACCGTAACA CGCCACATCT TGCGAATATA  
 TCCCTTTATC CGGTCCAAAA GTGGCATTGT GCGGTGTAGA ACGCTTATAT  
 4351 TGTGTAGAAA CTGCCGGAAT TCGTCGTGGT ATTCACTCCA GAGCGATGAA  
 ACACATCTTT GACGGCCTTT AGCAGCACCA TAAGTGAGGT CTCGCTACTT  
 4401 AACGTTTCAG TTTGCTCATG GAAAACGGTG TAACAAGGGT GAACACTATC  
 TTGCAAAGTC AAACGAGTAC CTTTGGCCAC ATTGTTCCCA CTTGTGATAG  
 4451 CCATATCACC AGCTCACCCT CTTTCATTGC CATACGGAAC TCCGGGTGAG  
 GGTATAGTGG TCGAGTGGCA GAAAGTAACG GTATGCCTTG AGGCCCACTC

## Fig. 3 cont.

4501 CATTCATCAG GCGGGCAAGA ATGTGAATAA AGGCCGGATA AAAC TTGTGC  
 GTAAGTAGTC CGCCCGTTCT TACACTTATT TCCGGCCTAT TTTGAACACG

4551 TTATTTTCTT TTACGGTCTT TAAAAAGGCC GTAATATCCA GCTGAACGGT  
 AATAAAAAGA AATGCCAGAA ATTTTTCCTG CATTATAGGT CGACTTGCCA

4601 CTGGTTATAG GTACATTGAG CAACTGACTG AAATGCCTCA AAATGTTCTT  
 GACCAATATC CATGTAATC GTTGACTGAC TTTACGGAGT TTTACAAGAA

4651 TACGATGCCA TTGGGATATA TCAACGGTGG TATATCCAGT GATTTTTTTC  
 ATGCTACGGT AACCTTATAT AGTTGCCACC ATATAGGTCA CTAAAAAAG

4701 TCCATTTTAG CTTCTTAGC TCCTGAAAAT CTCGATAACT CAAAAAATAC  
 AGGTAAAATC GAAGGAATCG AGGACTTTTA GAGCTATTGA GTTTTTTATG

4751 GCCCGGTAGT GATCTTATTT CATTATGGTG AAAGTTGGAA CCTCACC CGA  
 CGGGCCATCA CTAGAATAAA GTAATACCAC TTTCAACCTT GGAGTGGGCT

4801 CGTCTAATGT GAGTTAGCTC ACTCATTAGG CACCCCAGGC TTTACACTTT  
 GCAGATTACA CTCAATCGAG TGAGTAATCC GTGGGGTCCG AAATGTGAAA

4851 ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAAATTTCA  
 TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT

M13 Reverse primer 100.0% XbaI  
 =====

4901 CACAGGAAAC AGCTATGACC ATGATTACGA ATTTCTAGAT AACGAGGGCA  
 GTGTCCTTTG TCGATACTGG TACTAATGCT TAAAGATCTA TTGCTCCCGT

4951 AAAAATGAAA AAGACAGCTA TCGCGATTGC AGTGGCACTG GCTGGTTTCG  
 TTTTACTTT TTCTGTCGAT AGCGCTAACG TCACCGTGAC CGACCAAAGC

EcoRV  
 ~~~~

5001 CTACCGTAGC GCAGGCCGAT  
 GATGGCATCG CGTCCGGCTA

Fig. 4a

## Sequence of MS-Roche#3, #7 and #8

VL

| Framework 1 |       |   |   |   |   |   |   |   |   |   |       |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   | Framework 2 |      |   |   |   |   |   |   |  |  |  |      |  |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  | CDR2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|-------------|-------|---|---|---|---|---|---|---|---|---|-------|---|---|---|---|---|---|---|---|---|------|---|---|---|---|---|---|---|---|-------------|------|---|---|---|---|---|---|--|--|--|------|--|--|--|--|--|--|--|--|--|------|--|--|--|--|--|--|--|--|------|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|---|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|---|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|--|
| 1           |       |   |   |   |   |   |   |   |   |   |       |   |   |   |   |   |   |   |   |   |      |   |   |   |   |   |   |   |   | 2           |      |   |   |   |   |   |   |  |  |  |      |  |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  | 3    |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 4 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Position    | 1     | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1     | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1    | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0           | 1    | 2 | 3 | 4 | 5 | 6 | 7 |  |  |  |      |  |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|             | EcoRV |   |   |   |   |   |   |   |   |   | BamHI |   |   |   |   |   |   |   |   |   | PstI |   |   |   |   |   |   |   |   |             | KpnI |   |   |   |   |   |   |  |  |  | SmaI |  |  |  |  |  |  |  |  |  | AseI |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| V Lc3       | D     | I | V | L | T | Q | S | P | A | T | L     | S | L | S | P | G | E | R | A | T | L    | S | C | R | A | S | S | S | S | S           | S    | S | S | S | S | S | S |  |  |  |      |  |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MS-Roché #3 | D     | I | V | L | T | Q | S | P | A | T | L     | S | L | S | P | G | E | R | A | T | L    | S | C | R | A | S | S | S | S | S           | S    | S | S | S | S | S | S |  |  |  |      |  |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MS-Roché #7 | D     | I | V | L | T | Q | S | P | A | T | L     | S | L | S | P | G | E | R | A | T | L    | S | C | R | A | S | S | S | S | S           | S    | S | S | S | S | S | S |  |  |  |      |  |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MS-Roché #8 | D     | I | V | L | T | Q | S | P | A | T | L     | S | L | S | P | G | E | R | A | T | L    | S | C | R | A | S | S | S | S | S           | S    | S | S | S | S | S | S |  |  |  |      |  |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |      |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

VL#3

MS-Roche #3

MS-Roche #7

MS-Roche #8

VH

| Framework 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | Framework 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | CDR2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   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  |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   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|   |   |   |   |   |   |
| 1           | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1           | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1    | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 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| Framework 3   |  |  |  |  |  |  |  |  |  | Framework 4   |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |
|---|--|--|--|--|--|--|--|--|--|---|--|--|--|--|--|--|--|--|--|---|--|--|--|--|--|--|--|--|--|---|--|--|--|--|--|--|--|--|--|
| 6   |  |  |  |  |  |  |  |  |  | 9   |  |  |  |  |  |  |  |  |  | 10  |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |
| 7   |  |  |  |  |  |  |  |  |  | 8   |  |  |  |  |  |  |  |  |  | MscI  |  |  |  |  |  |  |  |  |  | BstXI   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
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| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
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| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9                   |  |  |  |  |  |  |  |  |  |
| BamHI   |  |  |  |  |  |  |  |  |  | BbsI  |  |  |  |  |  |  |  |  |  | T F G Q   |  |  |  |  |  |  |  |  |  | G T K V E I K R T   |  |  |  |  |  |  |  |  |  |
| V P A R F S G S G S G T D F T L T I S S L E P E D F A T W Y Y C                     |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C                       |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C   |  |  |  |  |  |  |  |  |  | V P A R F S G S G S G T D F T L T I S S L E P E D F A T Y Y C |  |  |  |  |  |  |  |  |  |
| 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  | 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 |  |  |  |  |  |  |  |  |  |   |  |  |  |  |  |  |  |  |  |

| Framework 3 |  |  |  |  |  |  |  |  |  | Framework 4 |  |  |  |  |  |  |  |  |  |
|-------------|--|--|--|--|--|--|--|--|--|-------------|--|--|--|--|--|--|--|--|--|
| 6           |  |  |  |  |  |  |  |  |  | 11          |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 10          |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 10          |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 11          |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 12          |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 13          |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 14          |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 15          |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 16          |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 17          |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 18          |  |  |  |  |  |  |  |  |  | -1          |  |  |  |  |  |  |  |  |  |
| 19          |  |  |  |  |  |  |  |  |  | -2          |  |  |  |  |  |  |  |  |  |
| 20          |  |  |  |  |  |  |  |  |  | -3          |  |  |  |  |  |  |  |  |  |
| 21          |  |  |  |  |  |  |  |  |  | -4          |  |  |  |  |  |  |  |  |  |
| 22          |  |  |  |  |  |  |  |  |  | -5          |  |  |  |  |  |  |  |  |  |
| 23          |  |  |  |  |  |  |  |  |  | -6          |  |  |  |  |  |  |  |  |  |
| 24          |  |  |  |  |  |  |  |  |  | -7          |  |  |  |  |  |  |  |  |  |
| 25          |  |  |  |  |  |  |  |  |  | -8          |  |  |  |  |  |  |  |  |  |
| 26          |  |  |  |  |  |  |  |  |  | -9          |  |  |  |  |  |  |  |  |  |
| 27          |  |  |  |  |  |  |  |  |  | -10         |  |  |  |  |  |  |  |  |  |
| 28          |  |  |  |  |  |  |  |  |  | -11         |  |  |  |  |  |  |  |  |  |
| 29          |  |  |  |  |  |  |  |  |  | -12         |  |  |  |  |  |  |  |  |  |
| 30          |  |  |  |  |  |  |  |  |  | -13         |  |  |  |  |  |  |  |  |  |
| 31          |  |  |  |  |  |  |  |  |  | -14         |  |  |  |  |  |  |  |  |  |
| 32          |  |  |  |  |  |  |  |  |  | -15         |  |  |  |  |  |  |  |  |  |
| 33          |  |  |  |  |  |  |  |  |  | -16         |  |  |  |  |  |  |  |  |  |
| 34          |  |  |  |  |  |  |  |  |  | -17         |  |  |  |  |  |  |  |  |  |
| 35          |  |  |  |  |  |  |  |  |  | -18         |  |  |  |  |  |  |  |  |  |
| 36          |  |  |  |  |  |  |  |  |  | -19         |  |  |  |  |  |  |  |  |  |
| 37          |  |  |  |  |  |  |  |  |  | -20         |  |  |  |  |  |  |  |  |  |
| 38          |  |  |  |  |  |  |  |  |  | -21         |  |  |  |  |  |  |  |  |  |
| 39          |  |  |  |  |  |  |  |  |  | -22         |  |  |  |  |  |  |  |  |  |
| 40          |  |  |  |  |  |  |  |  |  | -23         |  |  |  |  |  |  |  |  |  |
| 41          |  |  |  |  |  |  |  |  |  | -24         |  |  |  |  |  |  |  |  |  |
| 42          |  |  |  |  |  |  |  |  |  | -25         |  |  |  |  |  |  |  |  |  |
| 43          |  |  |  |  |  |  |  |  |  | -26         |  |  |  |  |  |  |  |  |  |
| 44          |  |  |  |  |  |  |  |  |  | -27         |  |  |  |  |  |  |  |  |  |
| 45          |  |  |  |  |  |  |  |  |  | -28         |  |  |  |  |  |  |  |  |  |
| 46          |  |  |  |  |  |  |  |  |  | -29         |  |  |  |  |  |  |  |  |  |
| 47          |  |  |  |  |  |  |  |  |  | -30         |  |  |  |  |  |  |  |  |  |
| 48          |  |  |  |  |  |  |  |  |  | -31         |  |  |  |  |  |  |  |  |  |
| 49          |  |  |  |  |  |  |  |  |  | -32         |  |  |  |  |  |  |  |  |  |
| 50          |  |  |  |  |  |  |  |  |  | -33         |  |  |  |  |  |  |  |  |  |
| 51          |  |  |  |  |  |  |  |  |  | -34         |  |  |  |  |  |  |  |  |  |
| 52          |  |  |  |  |  |  |  |  |  | -35         |  |  |  |  |  |  |  |  |  |
| 53          |  |  |  |  |  |  |  |  |  | -36         |  |  |  |  |  |  |  |  |  |
| 54          |  |  |  |  |  |  |  |  |  | -37         |  |  |  |  |  |  |  |  |  |
| 55          |  |  |  |  |  |  |  |  |  | -38         |  |  |  |  |  |  |  |  |  |
| 56          |  |  |  |  |  |  |  |  |  | -39         |  |  |  |  |  |  |  |  |  |
| 57          |  |  |  |  |  |  |  |  |  | -40         |  |  |  |  |  |  |  |  |  |
| 58          |  |  |  |  |  |  |  |  |  | -41         |  |  |  |  |  |  |  |  |  |
| 59          |  |  |  |  |  |  |  |  |  | -42         |  |  |  |  |  |  |  |  |  |
| 60          |  |  |  |  |  |  |  |  |  | -43         |  |  |  |  |  |  |  |  |  |
| 61          |  |  |  |  |  |  |  |  |  | -44         |  |  |  |  |  |  |  |  |  |
| 62          |  |  |  |  |  |  |  |  |  | -45         |  |  |  |  |  |  |  |  |  |
| 63          |  |  |  |  |  |  |  |  |  | -46         |  |  |  |  |  |  |  |  |  |
| 64          |  |  |  |  |  |  |  |  |  | -47         |  |  |  |  |  |  |  |  |  |
| 65          |  |  |  |  |  |  |  |  |  | -48         |  |  |  |  |  |  |  |  |  |
| 66          |  |  |  |  |  |  |  |  |  | -49         |  |  |  |  |  |  |  |  |  |
| 67          |  |  |  |  |  |  |  |  |  | -50         |  |  |  |  |  |  |  |  |  |
| 68          |  |  |  |  |  |  |  |  |  | -51         |  |  |  |  |  |  |  |  |  |
| 69          |  |  |  |  |  |  |  |  |  | -52         |  |  |  |  |  |  |  |  |  |
| 70          |  |  |  |  |  |  |  |  |  | -53         |  |  |  |  |  |  |  |  |  |
| 71          |  |  |  |  |  |  |  |  |  | -54         |  |  |  |  |  |  |  |  |  |
| 72          |  |  |  |  |  |  |  |  |  | -55         |  |  |  |  |  |  |  |  |  |
| 73          |  |  |  |  |  |  |  |  |  | -56         |  |  |  |  |  |  |  |  |  |
| 74          |  |  |  |  |  |  |  |  |  | -57         |  |  |  |  |  |  |  |  |  |
| 75          |  |  |  |  |  |  |  |  |  | -58         |  |  |  |  |  |  |  |  |  |
| 76          |  |  |  |  |  |  |  |  |  | -59         |  |  |  |  |  |  |  |  |  |
| 77          |  |  |  |  |  |  |  |  |  | -60         |  |  |  |  |  |  |  |  |  |
| 78          |  |  |  |  |  |  |  |  |  | -61         |  |  |  |  |  |  |  |  |  |
| 79          |  |  |  |  |  |  |  |  |  | -62         |  |  |  |  |  |  |  |  |  |
| 80          |  |  |  |  |  |  |  |  |  | -63         |  |  |  |  |  |  |  |  |  |
| 81          |  |  |  |  |  |  |  |  |  | -64         |  |  |  |  |  |  |  |  |  |
| 82          |  |  |  |  |  |  |  |  |  | -65         |  |  |  |  |  |  |  |  |  |
| 83          |  |  |  |  |  |  |  |  |  | -66         |  |  |  |  |  |  |  |  |  |
| 84          |  |  |  |  |  |  |  |  |  | -67         |  |  |  |  |  |  |  |  |  |
| 85          |  |  |  |  |  |  |  |  |  | -68         |  |  |  |  |  |  |  |  |  |
| 86          |  |  |  |  |  |  |  |  |  | -69         |  |  |  |  |  |  |  |  |  |
| 87          |  |  |  |  |  |  |  |  |  | -70         |  |  |  |  |  |  |  |  |  |
| 88          |  |  |  |  |  |  |  |  |  | -71         |  |  |  |  |  |  |  |  |  |
| 89          |  |  |  |  |  |  |  |  |  | -72         |  |  |  |  |  |  |  |  |  |
| 90          |  |  |  |  |  |  |  |  |  | -73         |  |  |  |  |  |  |  |  |  |
| 91          |  |  |  |  |  |  |  |  |  | -74         |  |  |  |  |  |  |  |  |  |
| 92          |  |  |  |  |  |  |  |  |  | -75         |  |  |  |  |  |  |  |  |  |
| 93          |  |  |  |  |  |  |  |  |  | -76         |  |  |  |  |  |  |  |  |  |
| 94          |  |  |  |  |  |  |  |  |  | -77         |  |  |  |  |  |  |  |  |  |
| 95          |  |  |  |  |  |  |  |  |  | -78         |  |  |  |  |  |  |  |  |  |
| 96          |  |  |  |  |  |  |  |  |  | -79         |  |  |  |  |  |  |  |  |  |
| 97          |  |  |  |  |  |  |  |  |  | -80         |  |  |  |  |  |  |  |  |  |
| 98          |  |  |  |  |  |  |  |  |  | -81         |  |  |  |  |  |  |  |  |  |
| 99          |  |  |  |  |  |  |  |  |  | -82         |  |  |  |  |  |  |  |  |  |
| 100         |  |  |  |  |  |  |  |  |  | -83         |  |  |  |  |  |  |  |  |  |
| 101         |  |  |  |  |  |  |  |  |  | -84         |  |  |  |  |  |  |  |  |  |
| 102         |  |  |  |  |  |  |  |  |  | -85         |  |  |  |  |  |  |  |  |  |
| 103         |  |  |  |  |  |  |  |  |  | -86         |  |  |  |  |  |  |  |  |  |
| 104         |  |  |  |  |  |  |  |  |  | -87         |  |  |  |  |  |  |  |  |  |
| 105         |  |  |  |  |  |  |  |  |  | -88         |  |  |  |  |  |  |  |  |  |
| 106         |  |  |  |  |  |  |  |  |  | -89         |  |  |  |  |  |  |  |  |  |
| 107         |  |  |  |  |  |  |  |  |  | -90         |  |  |  |  |  |  |  |  |  |
| 108         |  |  |  |  |  |  |  |  |  | -91         |  |  |  |  |  |  |  |  |  |
| 109         |  |  |  |  |  |  |  |  |  | -92         |  |  |  |  |  |  |  |  |  |
| 110         |  |  |  |  |  |  |  |  |  | -93         |  |  |  |  |  |  |  |  |  |
| 111         |  |  |  |  |  |  |  |  |  | -94         |  |  |  |  |  |  |  |  |  |
| 112         |  |  |  |  |  |  |  |  |  | -95         |  |  |  |  |  |  |  |  |  |
| 113         |  |  |  |  |  |  |  |  |  | -96         |  |  |  |  |  |  |  |  |  |
| 114         |  |  |  |  |  |  |  |  |  | -97         |  |  |  |  |  |  |  |  |  |
| 115         |  |  |  |  |  |  |  |  |  | -98         |  |  |  |  |  |  |  |  |  |
| 116         |  |  |  |  |  |  |  |  |  | -99         |  |  |  |  |  |  |  |  |  |
| 117         |  |  |  |  |  |  |  |  |  | -100        |  |  |  |  |  |  |  |  |  |
| 118         |  |  |  |  |  |  |  |  |  | -101        |  |  |  |  |  |  |  |  |  |
| 119         |  |  |  |  |  |  |  |  |  | -102        |  |  |  |  |  |  |  |  |  |
| 120         |  |  |  |  |  |  |  |  |  | -103        |  |  |  |  |  |  |  |  |  |
| 121         |  |  |  |  |  |  |  |  |  | -104        |  |  |  |  |  |  |  |  |  |
| 122         |  |  |  |  |  |  |  |  |  | -105        |  |  |  |  |  |  |  |  |  |
| 123         |  |  |  |  |  |  |  |  |  | -106        |  |  |  |  |  |  |  |  |  |
| 124         |  |  |  |  |  |  |  |  |  | -107        |  |  |  |  |  |  |  |  |  |
| 125         |  |  |  |  |  |  |  |  |  | -108        |  |  |  |  |  |  |  |  |  |
| 126         |  |  |  |  |  |  |  |  |  | -109        |  |  |  |  |  |  |  |  |  |
| 127         |  |  |  |  |  |  |  |  |  | -110        |  |  |  |  |  |  |  |  |  |
| 128         |  |  |  |  |  |  |  |  |  | -111        |  |  |  |  |  |  |  |  |  |
| 129         |  |  |  |  |  |  |  |  |  | -112        |  |  |  |  |  |  |  |  |  |
| 130         |  |  |  |  |  |  |  |  |  | -113        |  |  |  |  |  |  |  |  |  |
| 131         |  |  |  |  |  |  |  |  |  | -114        |  |  |  |  |  |  |  |  |  |
| 132         |  |  |  |  |  |  |  |  |  | -115        |  |  |  |  |  |  |  |  |  |
| 133         |  |  |  |  |  |  |  |  |  | -116        |  |  |  |  |  |  |  |  |  |
| 134         |  |  |  |  |  |  |  |  |  | -117        |  |  |  |  |  |  |  |  |  |
| 135         |  |  |  |  |  |  |  |  |  | -118        |  |  |  |  |  |  |  |  |  |
| 136         |  |  |  |  |  |  |  |  |  | -119        |  |  |  |  |  |  |  |  |  |
| 137         |  |  |  |  |  |  |  |  |  | -120        |  |  |  |  |  |  |  |  |  |
| 138         |  |  |  |  |  |  |  |  |  | -121        |  |  |  |  |  |  |  |  |  |
| 139         |  |  |  |  |  |  |  |  |  | -122        |  |  |  |  |  |  |  |  |  |
| 140         |  |  |  |  |  |  |  |  |  | -123        |  |  |  |  |  |  |  |  |  |
| 141         |  |  |  |  |  |  |  |  |  | -124        |  |  |  |  |  |  |  |  |  |
| 142         |  |  |  |  |  |  |  |  |  | -125        |  |  |  |  |  |  |  |  |  |
| 143         |  |  |  |  |  |  |  |  |  | -126        |  |  |  |  |  |  |  |  |  |
| 144         |  |  |  |  |  |  |  |  |  | -127        |  |  |  |  |  |  |  |  |  |
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| 146         |  |  |  |  |  |  |  |  |  | -129        |  |  |  |  |  |  |  |  |  |
| 147         |  |  |  |  |  |  |  |  |  | -130        |  |  |  |  |  |  |  |  |  |
| 148         |  |  |  |  |  |  |  |  |  | -131        |  |  |  |  |  |  |  |  |  |
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| 150         |  |  |  |  |  |  |  |  |  | -133        |  |  |  |  |  |  |  |  |  |
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| 154         |  |  |  |  |  |  |  |  |  | -137        |  |  |  |  |  |  |  |  |  |
| 155         |  |  |  |  |  |  |  |  |  | -138        |  |  |  |  |  |  |  |  |  |
| 156         |  |  |  |  |  |  |  |  |  | -139        |  |  |  |  |  |  |  |  |  |
| 157         |  |  |  |  |  |  |  |  |  | -140        |  |  |  |  |  |  |  |  |  |
| 158         |  |  |  |  |  |  |  |  |  | -141        |  |  |  |  |  |  |  |  |  |
| 159         |  |  |  |  |  |  |  |  |  | -142        |  |  |  |  |  |  |  |  |  |
| 160         |  |  |  |  |  |  |  |  |  | -143        |  |  |  |  |  |  |  |  |  |
| 161         |  |  |  |  |  |  |  |  |  | -144        |  |  |  |  |  |  |  |  |  |
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| 163         |  |  |  |  |  |  |  |  |  | -146        |  |  |  |  |  |  |  |  |  |
| 164         |  |  |  |  |  |  |  |  |  | -147        |  |  |  |  |  |  |  |  |  |
| 165         |  |  |  |  |  |  |  |  |  | -148        |  |  |  |  |  |  |  |  |  |
| 166         |  |  |  |  |  |  |  |  |  | -149        |  |  |  |  |  |  |  |  |  |
| 167         |  |  |  |  |  |  |  |  |  | -150        |  |  |  |  |  |  |  |  |  |
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| 169         |  |  |  |  |  |  |  |  |  | -152        |  |  |  |  |  |  |  |  |  |
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| 172         |  |  |  |  |  |  |  |  |  | -155        |  |  |  |  |  |  |  |  |  |
| 173         |  |  |  |  |  |  |  |  |  | -156        |  |  |  |  |  |  |  |  |  |
| 174         |  |  |  |  |  |  |  |  |  | -157        |  |  |  |  |  |  |  |  |  |
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| 178         |  |  |  |  |  |  |  |  |  | -161        |  |  |  |  |  |  |  |  |  |
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| 180         |  |  |  |  |  |  |  |  |  | -163        |  |  |  |  |  |  |  |  |  |
| 181         |  |  |  |  |  |  |  |  |  | -164        |  |  |  |  |  |  |  |  |  |
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| 184         |  |  |  |  |  |  |  |  |  | -167        |  |  |  |  |  |  |  |  |  |
| 185         |  |  |  |  |  |  |  |  |  | -168        |  |  |  |  |  |  |  |  |  |
| 186         |  |  |  |  |  |  |  |  |  | -169        |  |  |  |  |  |  |  |  |  |
| 187         |  |  |  |  |  |  |  |  |  | -170        |  |  |  |  |  |  |  |  |  |
| 188         |  |  |  |  |  |  |  |  |  | -171        |  |  |  |  |  |  |  |  |  |
| 189         |  |  |  |  |  |  |  |  |  | -172        |  |  |  |  |  |  |  |  |  |
| 190         |  |  |  |  |  |  |  |  |  | -173        |  |  |  |  |  |  |  |  |  |
| 191         |  |  |  |  |  |  |  |  |  | -174        |  |  |  |  |  |  |  |  |  |
| 192         |  |  |  |  |  |  |  |  |  | -175        |  |  |  |  |  |  |  |  |  |
| 193         |  |  |  |  |  |  |  |  |  | -176        |  |  |  |  |  |  |  |  |  |
| 194         |  |  |  |  |  |  |  |  |  | -177        |  |  |  |  |  |  |  |  |  |
| 195         |  |  |  |  |  |  |  |  |  | -178        |  |  |  |  |  |  |  |  |  |
| 196         |  |  |  |  |  |  |  |  |  | -179        |  |  |  |  |  |  |  |  |  |
| 197         |  |  |  |  |  |  |  |  |  | -180        |  |  |  |  |  |  |  |  |  |
| 198         |  |  |  |  |  |  |  |  |  | -181        |  |  |  |  |  |  |  |  |  |
| 199         |  |  |  |  |  |  |  |  |  | -182        |  |  |  |  |  |  |  |  |  |
| 200         |  |  |  |  |  |  |  |  |  | -183        |  |  |  |  |  |  |  |  |  |
| 201         |  |  |  |  |  |  |  |  |  | -184        |  |  |  |  |  |  |  |  |  |
| 202         |  |  |  |  |  |  |  |  |  | -185        |  |  |  |  |  |  |  |  |  |
| 203         |  |  |  |  |  |  |  |  |  | -186        |  |  |  |  |  |  |  |  |  |
| 204         |  |  |  |  |  |  |  |  |  | -187        |  |  |  |  |  |  |  |  |  |
| 205         |  |  |  |  |  |  |  |  |  | -188        |  |  |  |  |  |  |  |  |  |
| 206         |  |  |  |  |  |  |  |  |  | -189        |  |  |  |  |  |  |  |  |  |
| 207         |  |  |  |  |  |  |  |  |  | -190        |  |  |  |  |  |  |  |  |  |
| 208         |  |  |  |  |  |  |  |  |  | -191        |  |  |  |  |  |  |  |  |  |
| 209         |  |  |  |  |  |  |  |  |  | -192        |  |  |  |  |  |  |  |  |  |
| 210         |  |  |  |  |  |  |  |  |  | -193        |  |  |  |  |  |  |  |  |  |
| 211         |  |  |  |  |  |  |  |  |  | -194        |  |  |  |  |  |  |  |  |  |
| 212         |  |  |  |  |  |  |  |  |  | -195        |  |  |  |  |  |  |  |  |  |
| 213         |  |  |  |  |  |  |  |  |  | -196        |  |  |  |  |  |  |  |  |  |
| 214         |  |  |  |  |  |  |  |  |  | -197        |  |  |  |  |  |  |  |  |  |
| 215         |  |  |  |  |  |  |  |  |  | -198        |  |  |  |  |  |  |  |  |  |
| 216         |  |  |  |  |  |  |  |  |  | -199        |  |  |  |  |  |  |  |  |  |
| 217         |  |  |  |  |  |  |  |  |  | -200        |  |  |  |  |  |  |  |  |  |
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| 222         |  |  |  |  |  |  |  |  |  | -205        |  |  |  |  |  |  |  |  |  |
| 223         |  |  |  |  |  |  |  |  |  | -206        |  |  |  |  |  |  |  |  |  |
| 224         |  |  |  |  |  |  |  |  |  | -207        |  |  |  |  |  |  |  |  |  |
| 225         |  |  |  |  |  |  |  |  |  | -208        |  |  |  |  |  |  |  |  |  |
| 226         |  |  |  |  |  |  |  |  |  | -209        |  |  |  |  |  |  |  |  |  |
| 227         |  |  |  |  |  |  |  |  |  | -210        |  |  |  |  |  |  |  |  |  |
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| 231         |  |  |  |  |  |  |  |  |  | -214        |  |  |  |  |  |  |  |  |  |
| 232         |  |  |  |  |  |  |  |  |  | -215        |  |  |  |  |  |  |  |  |  |
| 233         |  |  |  |  |  |  |  |  |  | -216        |  |  |  |  |  |  |  |  |  |
| 234         |  |  |  |  |  |  |  |  |  | -217        |  |  |  |  |  |  |  |  |  |
| 235         |  |  |  |  |  |  |  |  |  | -218        |  |  |  |  |  |  |  |  |  |
| 236         |  |  |  |  |  |  |  |  |  | -219        |  |  |  |  |  |  |  |  |  |
| 237         |  |  |  |  |  |  |  |  |  | -220        |  |  |  |  |  |  |  |  |  |
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| 243         |  |  |  |  |  |  |  |  |  | -226        |  |  |  |  |  |  |  |  |  |
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| 283         |  |  |  |  |  |  |  |  |  | -266        |  |  |  |  |  |  |  |  |  |
| 284         |  |  |  |  |  |  |  |  |  | -267        |  |  |  |  |  |  |  |  |  |
| 285         |  |  |  |  |  |  |  |  |  | -268        |  |  |  |  |  |  |  |  |  |
| 286         |  |  |  |  |  |  |  |  |  | -269        |  |  |  |  |  |  |  |  |  |
| 287         |  |  |  |  |  |  |  |  |  | -270        |  |  |  |  |  |  |  |  |  |
| 288         |  |  |  |  |  |  |  |  |  | -271        |  |  |  |  |  |  |  |  |  |
| 289         |  |  |  |  |  |  |  |  |  | -272        |  |  |  |  |  |  |  |  |  |
| 290         |  |  |  |  |  |  |  |  |  | -273        |  |  |  |  |  |  |  |  |  |
| 291         |  |  |  |  |  |  |  |  |  | -274        |  |  |  |  |  |  |  |  |  |
| 292         |  |  |  |  |  |  |  |  |  | -275        |  |  |  |  |  |  |  |  |  |
| 293         |  |  |  |  |  |  |  |  |  | -276        |  |  |  |  |  |  |  |  |  |
| 294         |  |  |  |  |  |  |  |  |  | -277        |  |  |  |  |  |  |  |  |  |
| 295         |  |  |  |  |  |  |  |  |  | -278        |  |  |  |  |  |  |  |  |  |
| 296         |  |  |  |  |  |  |  |  |  | -279        |  |  |  |  |  |  |  |  |  |
| 297         |  |  |  |  |  |  |  |  |  | -280        |  |  |  |  |  |  |  |  |  |
| 298         |  |  |  |  |  |  |  |  |  | -281        |  |  |  |  |  |  |  |  |  |
| 299         |  |  |  |  |  |  |  |  |  | -282        |  |  |  |  |  |  |  |  |  |
| 300         |  |  |  |  |  |  |  |  |  | -283        |  |  |  |  |  |  |  |  |  |
| 301         |  |  |  |  |  |  |  |  |  | -284        |  |  |  |  |  |  |  |  |  |
| 302         |  |  |  |  |  |  |  |  |  | -285        |  |  |  |  |  |  |  |  |  |
| 303         |  |  |  |  |  |  |  |  |  | -286        |  |  |  |  |  |  |  |  |  |
| 304         |  |  |  |  |  |  |  |  |  | -287        |  |  |  |  |  |  |  |  |  |
| 305         |  |  |  |  |  |  |  |  |  | -288        |  |  |  |  |  |  |  |  |  |
| 306         |  |  |  |  |  |  |  |  |  | -289        |  |  |  |  |  |  |  |  |  |
| 307         |  |  |  |  |  |  |  |  |  | -290        |  |  |  |  |  |  |  |  |  |
| 308         |  |  |  |  |  |  |  |  |  | -291        |  |  |  |  |  |  |  |  |  |
| 309         |  |  |  |  |  |  |  |  |  | -292        |  |  |  |  |  |  |  |  |  |
| 310         |  |  |  |  |  |  |  |  |  | -293        |  |  |  |  |  |  |  |  |  |
| 311         |  |  |  |  |  |  |  |  |  | -294        |  |  |  |  |  |  |  |  |  |
| 312         |  |  |  |  |  |  |  |  |  | -295        |  |  |  |  |  |  |  |  |  |
| 313         |  |  |  |  |  |  |  |  |  | -296        |  |  |  |  |  |  |  |  |  |
| 314         |  |  |  |  |  |  |  |  |  | -297        |  |  |  |  |  |  |  |  |  |
| 315         |  |  |  |  |  |  |  |  |  | -298        |  |  |  |  |  |  |  |  |  |
| 316         |  |  |  |  |  |  |  |  |  | -299        |  |  |  |  |  |  |  |  |  |
| 317         |  |  |  |  |  |  |  |  |  | -300        |  |  |  |  |  |  |  |  |  |
| 318         |  |  |  |  |  |  |  |  |  | -301        |  |  |  |  |  |  |  |  |  |
| 319         |  |  |  |  |  |  |  |  |  | -302        |  |  |  |  |  |  |  |  |  |
| 320         |  |  |  |  |  |  |  |  |  | -303        |  |  |  |  |  |  |  |  |  |
| 321         |  |  |  |  |  |  |  |  |  | -304        |  |  |  |  |  |  |  |  |  |
| 322         |  |  |  |  |  |  |  |  |  | -305        |  |  |  |  |  |  |  |  |  |
| 323         |  |  |  |  |  |  |  |  |  | -306        |  |  |  |  |  |  |  |  |  |
| 324         |  |  |  |  |  |  |  |  |  | -307        |  |  |  |  |  |  |  |  |  |
| 325         |  |  |  |  |  |  |  |  |  | -308        |  |  |  |  |  |  |  |  |  |
| 326         |  |  |  |  |  |  |  |  |  | -309        |  |  |  |  |  |  |  |  |  |
| 327         |  |  |  |  |  |  |  |  |  | -310        |  |  |  |  |  |  |  |  |  |
| 328         |  |  |  |  |  |  |  |  |  | -311        |  |  |  |  |  |  |  |  |  |
| 329         |  |  |  |  |  |  |  |  |  | -312        |  |  |  |  |  |  |  |  |  |
| 330         |  |  |  |  |  |  |  |  |  | -313        |  |  |  |  |  |  |  |  |  |
| 331         |  |  |  |  |  |  |  |  |  | -314        |  |  |  |  |  |  |  |  |  |
| 332         |  |  |  |  |  |  |  |  |  | -315        |  |  |  |  |  |  |  |  |  |
| 333         |  |  |  |  |  |  |  |  |  | -316        |  |  |  |  |  |  |  |  |  |
| 334         |  |  |  |  |  |  |  |  |  | -317        |  |  |  |  |  |  |  |  |  |
| 335         |  |  |  |  |  |  |  |  |  | -318        |  |  |  |  |  |  |  |  |  |
| 336         |  |  |  |  |  |  |  |  |  | -319        |  |  |  |  |  |  |  |  |  |
| 337         |  |  |  |  |  |  |  |  |  | -320        |  |  |  |  |  |  |  |  |  |
| 338         |  |  |  |  |  |  |  |  |  | -321        |  |  |  |  |  |  |  |  |  |
| 339         |  |  |  |  |  |  |  |  |  | -322        |  |  |  |  |  |  |  |  |  |
| 340         |  |  |  |  |  |  |  |  |  | -323        |  |  |  |  |  |  |  |  |  |
| 341         |  |  |  |  |  |  |  |  |  | -324        |  |  |  |  |  |  |  |  |  |
| 342         |  |  |  |  |  |  |  |  |  | -325        |  |  |  |  |  |  |  |  |  |
| 343         |  |  |  |  |  |  |  |  |  | -326        |  |  |  |  |  |  |  |  |  |
| 344         |  |  |  |  |  |  |  |  |  | -327        |  |  |  |  |  |  |  |  |  |
| 345         |  |  |  |  |  |  |  |  |  | -328        |  |  |  |  |  |  |  |  |  |
| 346         |  |  |  |  |  |  |  |  |  | -329        |  |  |  |  |  |  |  |  |  |
| 347         |  |  |  |  |  |  |  |  |  | -330        |  |  |  |  |  |  |  |  |  |
| 348         |  |  |  |  |  |  |  |  |  | -331        |  |  |  |  |  |  |  |  |  |
| 349         |  |  |  |  |  |  |  |  |  | -332        |  |  |  |  |  |  |  |  |  |
| 350         |  |  |  |  |  |  |  |  |  | -333        |  |  |  |  |  |  |  |  |  |
| 351         |  |  |  |  |  |  |  |  |  | -334        |  |  |  |  |  |  |  |  |  |
| 352         |  |  |  |  |  |  |  |  |  | -335        |  |  |  |  |  |  |  |  |  |
| 353         |  |  |  |  |  |  |  |  |  | -336        |  |  |  |  |  |  |  |  |  |
| 354         |  |  |  |  |  |  |  |  |  | -337        |  |  |  |  |  |  |  |  |  |
| 355         |  |  |  |  |  |  |  |  |  | -338        |  |  |  |  |  |  |  |  |  |
| 356         |  |  |  |  |  |  |  |  |  | -339        |  |  |  |  |  |  |  |  |  |
| 357         |  |  |  |  |  |  |  |  |  | -340        |  |  |  |  |  |  |  |  |  |
| 358         |  |  |  |  |  |  |  |  |  | -341        |  |  |  |  |  |  |  |  |  |
| 359         |  |  |  |  |  |  |  |  |  | -342        |  |  |  |  |  |  |  |  |  |
| 360         |  |  |  |  |  |  |  |  |  | -343        |  |  |  |  |  |  |  |  |  |
| 361         |  |  |  |  |  |  |  |  |  | -344        |  |  |  |  |  |  |  |  |  |
| 362         |  |  |  |  |  |  |  |  |  | -345        |  |  |  |  |  |  |  |  |  |
| 363         |  |  |  |  |  |  |  |  |  | -346        |  |  |  |  |  |  |  |  |  |
| 364         |  |  |  |  |  |  |  |  |  | -347        |  |  |  |  |  |  |  |  |  |
| 365         |  |  |  |  |  |  |  |  |  | -348        |  |  |  |  |  |  |  |  |  |
| 366         |  |  |  |  |  |  |  |  |  | -349        |  |  |  |  |  |  |  |  |  |
| 367         |  |  |  |  |  |  |  |  |  | -350        |  |  |  |  |  |  |  |  |  |
| 368         |  |  |  |  |  |  |  |  |  | -351        |  |  |  |  |  |  |  |  |  |
| 369         |  |  |  |  |  |  |  |  |  | -352        |  |  |  |  |  |  |  |  |  |
| 370         |  |  |  |  |  |  |  |  |  | -353        |  |  |  |  |  |  |  |  |  |
| 371         |  |  |  |  |  |  |  |  |  | -354        |  |  |  |  |  |  |  |  |  |
| 372         |  |  |  |  |  |  |  |  |  | -355        |  |  |  |  |  |  |  |  |  |
| 373         |  |  |  |  |  |  |  |  |  | -356        |  |  |  |  |  |  |  |  |  |
| 374         |  |  |  |  |  |  |  |  |  | -357        |  |  |  |  |  |  |  |  |  |
| 375         |  |  |  |  |  |  |  |  |  | -358        |  |  |  |  |  |  |  |  |  |
| 376         |  |  |  |  |  |  |  |  |  | -359        |  |  |  |  |  |  |  |  |  |
| 377         |  |  |  |  |  |  |  |  |  | -360        |  |  |  |  |  |  |  |  |  |
| 378         |  |  |  |  |  |  |  |  |  | -361        |  |  |  |  |  |  |  |  |  |
| 379         |  |  |  |  |  |  |  |  |  | -362        |  |  |  |  |  |  |  |  |  |
| 380         |  |  |  |  |  |  |  |  |  | -363        |  |  |  |  |  |  |  |  |  |
| 381         |  |  |  |  |  |  |  |  |  | -364        |  |  |  |  |  |  |  |  |  |
| 382         |  |  |  |  |  |  |  |  |  | -365        |  |  |  |  |  |  |  |  |  |
| 383         |  |  |  |  |  |  |  |  |  | -366        |  |  |  |  |  |  |  |  |  |
| 384         |  |  |  |  |  |  |  |  |  | -367        |  |  |  |  |  |  |  |  |  |
| 385         |  |  |  |  |  |  |  |  |  | -368        |  |  |  |  |  |  |  |  |  |
| 386         |  |  |  |  |  |  |  |  |  | -369        |  |  |  |  |  |  |  |  |  |
| 387         |  |  |  |  |  |  |  |  |  | -370        |  |  |  |  |  |  |  |  |  |
| 388         |  |  |  |  |  |  |  |  |  | -371        |  |  |  |  |  |  |  |  |  |
| 389         |  |  |  |  |  |  |  |  |  | -372        |  |  |  |  |  |  |  |  |  |
| 390         |  |  |  |  |  |  |  |  |  | -373        |  |  |  |  |  |  |  |  |  |
| 391         |  |  |  |  |  |  |  |  |  | -374        |  |  |  |  |  |  |  |  |  |
| 392         |  |  |  |  |  |  |  |  |  | -375        |  |  |  |  |  |  |  |  |  |
| 393         |  |  |  |  |  |  |  |  |  | -376        |  |  |  |  |  |  |  |  |  |
| 394         |  |  |  |  |  |  |  |  |  | -377        |  |  |  |  |  |  |  |  |  |
| 395         |  |  |  |  |  |  |  |  |  | -378        |  |  |  |  |  |  |  |  |  |
| 396         |  |  |  |  |  |  |  |  |  | -379        |  |  |  |  |  |  |  |  |  |
| 397         |  |  |  |  |  |  |  |  |  | -380        |  |  |  |  |  |  |  |  |  |
| 398         |  |  |  |  |  |  |  |  |  | -381        |  |  |  |  |  |  |  |  |  |
| 399         |  |  |  |  |  |  |  |  |  | -382        |  |  |  |  |  |  |  |  |  |
| 400         |  |  |  |  |  |  |  |  |  | -383        |  |  |  |  |  |  |  |  |  |
| 401         |  |  |  |  |  |  |  |  |  | -384        |  |  |  |  |  |  |  |  |  |
| 402         |  |  |  |  |  |  |  |  |  | -385        |  |  |  |  |  |  |  |  |  |
| 403         |  |  |  |  |  |  |  |  |  | -386        |  |  |  |  |  |  |  |  |  |
| 404         |  |  |  |  |  |  |  |  |  | -387        |  |  |  |  |  |  |  |  |  |
| 405         |  |  |  |  |  |  |  |  |  | -388        |  |  |  |  |  |  |  |  |  |
| 406         |  |  |  |  |  |  |  |  |  | -389        |  |  |  |  |  |  |  |  |  |
| 407         |  |  |  |  |  |  |  |  |  | -390        |  |  |  |  |  |  |  |  |  |
| 408         |  |  |  |  |  |  |  |  |  | -391        |  |  |  |  |  |  |  |  |  |
| 409         |  |  |  |  |  |  |  |  |  | -392        |  |  |  |  |  |  |  |  |  |
| 410         |  |  |  |  |  |  |  |  |  | -393        |  |  |  |  |  |  |  |  |  |
| 411         |  |  |  |  |  |  |  |  |  | -394        |  |  |  |  |  |  |  |  |  |
| 412         |  |  |  |  |  |  |  |  |  | -395        |  |  |  |  |  |  |  |  |  |
| 413         |  |  |  |  |  |  |  |  |  | -396        |  |  |  |  |  |  |  |  |  |
| 414         |  |  |  |  |  |  |  |  |  | -397        |  |  |  |  |  |  |  |  |  |
| 415         |  |  |  |  |  |  |  |  |  | -398        |  |  |  |  |  |  |  |  |  |
| 416         |  |  |  |  |  |  |  |  |  | -399        |  |  |  |  |  |  |  |  |  |
| 417         |  |  |  |  |  |  |  |  |  | -400        |  |  |  |  |  |  |  |  |  |
| 418         |  |  |  |  |  |  |  |  |  | -401        |  |  |  |  |  |  |  |  |  |
| 419         |  |  |  |  |  |  |  |  |  | -402        |  |  |  |  |  |  |  |  |  |
| 420         |  |  |  |  |  |  |  |  |  | -403        |  |  |  |  |  |  |  |  |  |
| 421         |  |  |  |  |  |  |  |  |  | -404        |  |  |  |  |  |  |  |  |  |
| 422         |  |  |  |  |  |  |  |  |  | -405        |  |  |  |  |  |  |  |  |  |
| 423         |  |  |  |  |  |  |  |  |  | -406        |  |  |  |  |  |  |  |  |  |
| 424         |  |  |  |  |  |  |  |  |  | -407        |  |  |  |  |  |  |  |  |  |
| 425         |  |  |  |  |  |  |  |  |  | -408        |  |  |  |  |  |  |  |  |  |
| 426         |  |  |  |  |  |  |  |  |  | -409        |  |  |  |  |  |  |  |  |  |
| 427         |  |  |  |  |  |  |  |  |  | -410        |  |  |  |  |  |  |  |  |  |
| 428         |  |  |  |  |  |  |  |  |  | -411        |  |  |  |  |  |  |  |  |  |
| 429         |  |  |  |  |  |  |  |  |  | -412        |  |  |  |  |  |  |  |  |  |
| 430         |  |  |  |  |  |  |  |  |  | -413        |  |  |  |  |  |  |  |  |  |
| 431         |  |  |  |  |  |  |  |  |  | -414        |  |  |  |  |  |  |  |  |  |
| 432         |  |  |  |  |  |  |  |  |  | -415        |  |  |  |  |  |  |  |  |  |
| 433         |  |  |  |  |  |  |  |  |  | -416        |  |  |  |  |  |  |  |  |  |
| 434         |  |  |  |  |  |  |  |  |  | -417        |  |  |  |  |  |  |  |  |  |
| 435         |  |  |  |  |  |  |  |  |  | -418        |  |  |  |  |  |  |  |  |  |
| 436         |  |  |  |  |  |  |  |  |  | -419        |  |  |  |  |  |  |  |  |  |
| 437         |  |  |  |  |  |  |  |  |  | -420        |  |  |  |  |  |  |  |  |  |
| 438         |  |  |  |  |  |  |  |  |  | -421        |  |  |  |  |  |  |  |  |  |
| 439         |  |  |  |  |  |  |  |  |  | -422        |  |  |  |  |  |  |  |  |  |
|             |  |  |  |  |  |  |  |  |  |             |  |  |  |  |  |  |  |  |  |



**Fig. 4b**

### Sequence of MS-Roche#3, #7 and #8

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## HV

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Fig. 4b cont.

| Framework 2 |  |  |  |  |  |  |  |  |  | Framework 3 |  |  |  |  |  |  |  |  |  |
|-------------|--|--|--|--|--|--|--|--|--|-------------|--|--|--|--|--|--|--|--|--|
| CDR 2       |  |  |  |  |  |  |  |  |  | CDR 3       |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
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| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
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| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
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| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
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| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
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| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
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| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
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| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
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| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
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| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
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| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
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| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
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| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
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| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
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| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
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| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  | 5           |  |  |  |  |  |  |  |  |  |
| 8           |  |  |  |  |  |  |  |  |  | 4           |  |  |  |  |  |  |  |  |  |
| 9           |  |  |  |  |  |  |  |  |  | 3           |  |  |  |  |  |  |  |  |  |
| 0           |  |  |  |  |  |  |  |  |  | 2           |  |  |  |  |  |  |  |  |  |
| 1           |  |  |  |  |  |  |  |  |  | 1           |  |  |  |  |  |  |  |  |  |
| 2           |  |  |  |  |  |  |  |  |  | 0           |  |  |  |  |  |  |  |  |  |
| 3           |  |  |  |  |  |  |  |  |  | 9           |  |  |  |  |  |  |  |  |  |
| 4           |  |  |  |  |  |  |  |  |  | 8           |  |  |  |  |  |  |  |  |  |
| 5           |  |  |  |  |  |  |  |  |  | 7           |  |  |  |  |  |  |  |  |  |
| 6           |  |  |  |  |  |  |  |  |  | 6           |  |  |  |  |  |  |  |  |  |
| 7           |  |  |  |  |  |  |  |  |  |             |  |  |  |  |  |  |  |  |  |

[illegible]

Figure 5

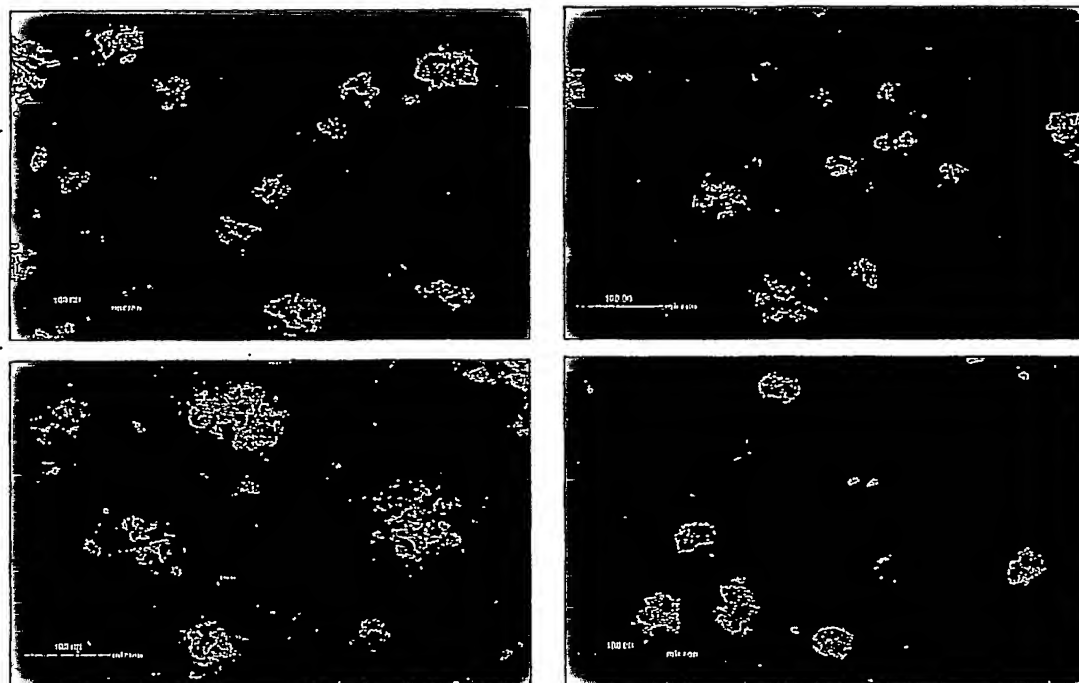


Figure 6

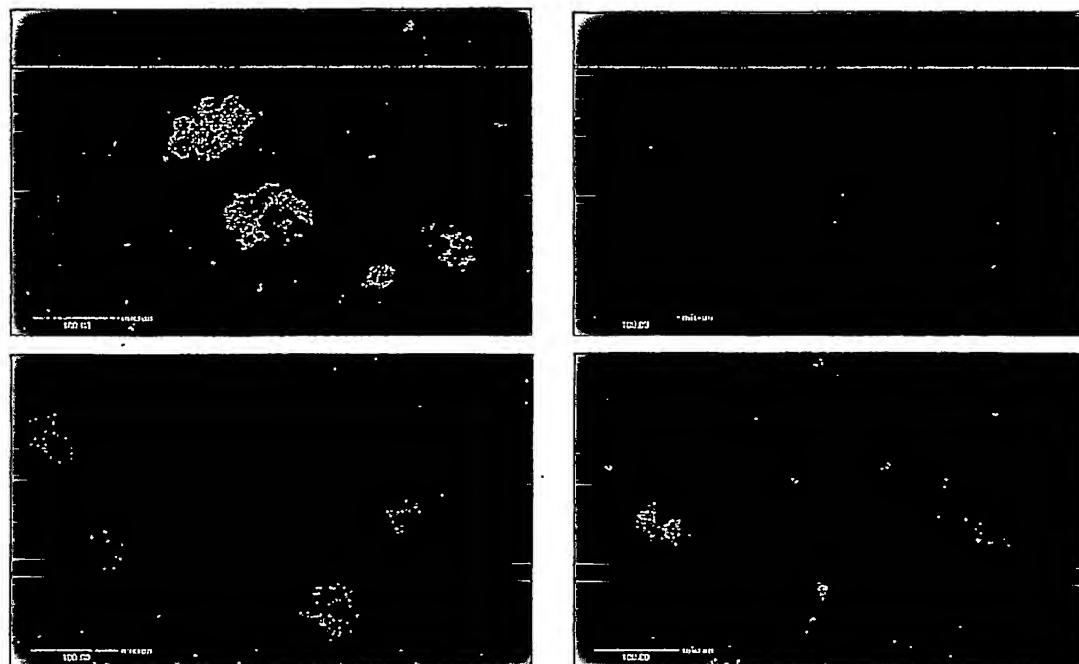
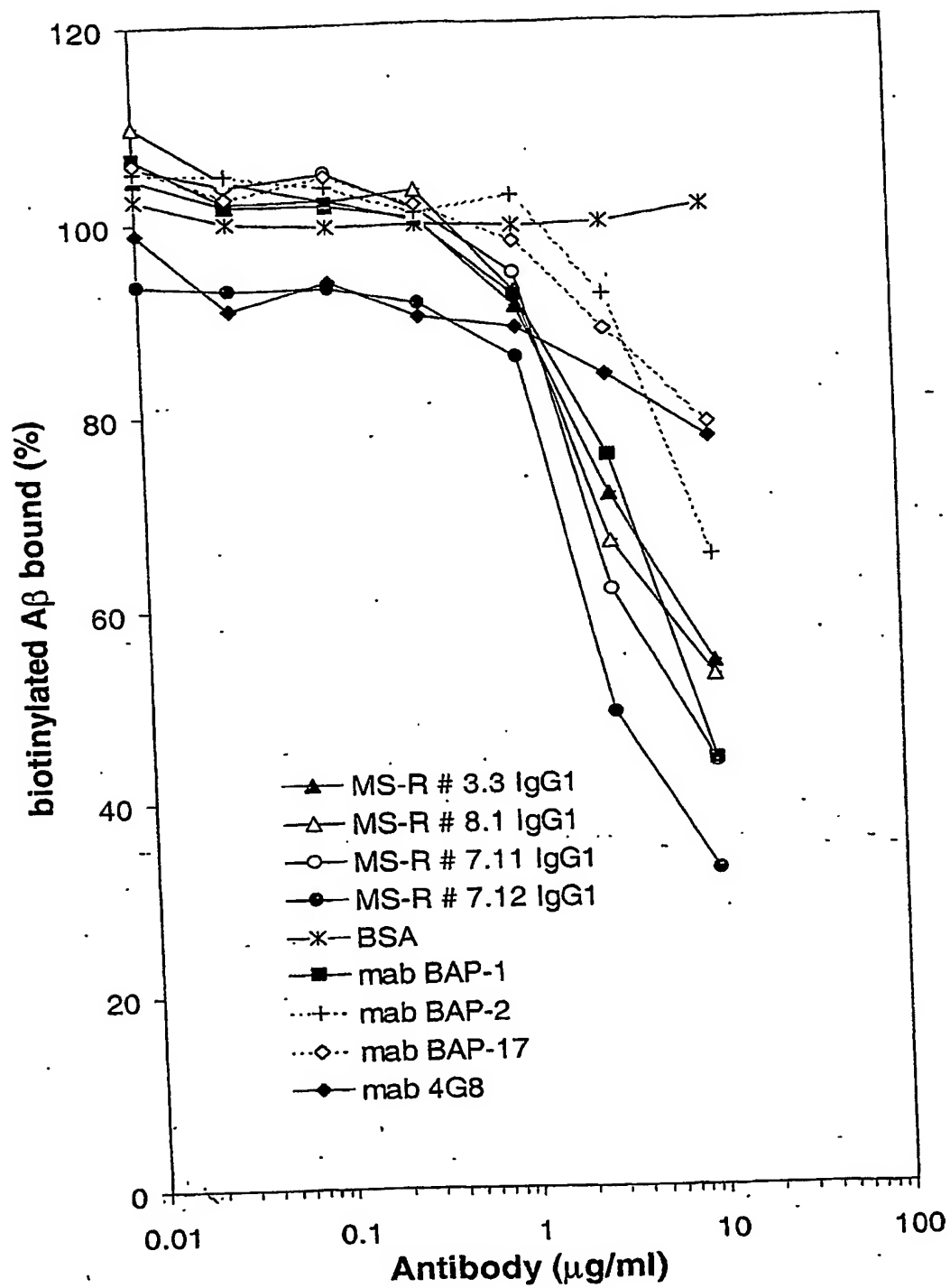
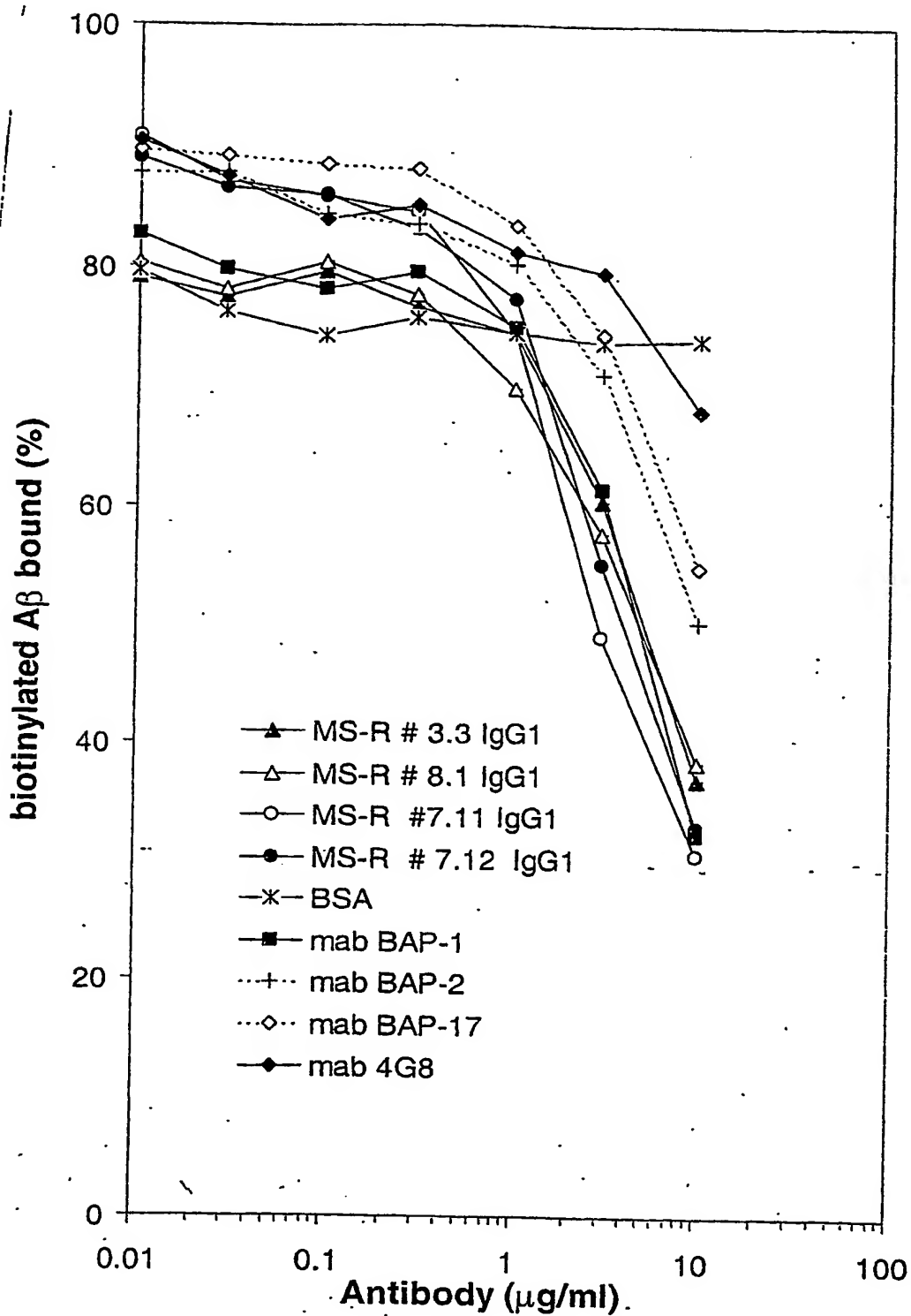


Fig. 7





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